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=> file biosis caba caplus embase lifesci medline scisearch
=> e carlsson jorgen/au
E1          33     CARLSSON JORG/AU
E2           9     CARLSSON JORG DR/AU
E3         247 --> CARLSSON JORGEN/AU
E4           1     CARLSSON JORGEN DR/AU
E5           3     CARLSSON JORGEN PROF/AU
E6         269     CARLSSON K/AU
E7           3     CARLSSON K A/AU
E8           6     CARLSSON K B/AU
E9           9     CARLSSON K C/AU
E10          3     CARLSSON K E/AU
E11          1     CARLSSON K G/AU
E12         70     CARLSSON K H/AU
=> s e1-e5 and (HER2 or SPA)
L1          42 ("CARLSSON JORG"/AU OR "CARLSSON JORG DR"/AU OR "CARLSSON JORGEN
               "/AU OR "CARLSSON JORGEN DR"/AU OR "CARLSSON JORGEN PROF"/AU)
               AND (HER2 OR SPA)
=> dup rem l1
PROCESSING COMPLETED FOR L1
L2          16 DUP REM L1 (26 DUPLICATES REMOVED)
=> d bib ab 1-
YOU HAVE REQUESTED DATA FROM 16 ANSWERS - CONTINUE? Y/(N):y

L2   ANSWER 1 OF 16  EMBASE  COPYRIGHT (c) 2009 Elsevier B.V. All rights
     reserved on STN                                        DUPLICATE 1
AN   2008123739  EMBASE   <<LOGINID::20090428>>
TI   EGFR,   ***HER2***  , and HER3 expression in laryngeal primary tumors and
     corresponding metastases.
AU   Wei, Qichun, Dr. (correspondence); Hu, Qiongge
CS   Department of Radiation Oncology, Second Affiliated Hospital, Hangzhou,
     310009, China. Qichun_Wei@zju.edu.cn
AU   Wei, Qichun, Dr. (correspondence); Sheng, Liming; Shui, Yongjie
CS   Cancer Institute, Zhejiang University, Hangzhou, 310009, China. Qichun_Wei
     @zju.edu.cn
AU   Wei, Qichun, Dr. (correspondence);   ***Carlsson, Jorgen***
CS   Department of Oncology, Radiology and Clinical Immunology, Rudbeck
     Laboratory, Uppsala University Hospital, SE-751 85, Uppsala, Sweden.
     Qichun_Wei@zju.edu.cn
AU   Nordgren, Hans
CS   Department of Genetics and Pathology, Rudbeck Laboratory, Uppsala
     University Hospital, SE-751 85, Uppsala, Sweden.
AU   Wei, Qichun, Dr. (correspondence); Hu, Qiongge
CS   Cancer Institute, Zhejiang University School of Medicine, Jiefang Road 88,
     Hangzhou, 310009, China. Qichun_Wei@zju.edu.cn
SO   Annals of Surgical Oncology, (Apr 2008) Vol. 15, No. 4, pp. 1193-1201.
     Refs: 41
     ISSN: 1068-9265  E-ISSN: 1534-4681  CODEN: ASONF4
CY   United States
DT   Journal; Conference Article; (Conference paper)
FS   011      Otorhinolaryngology
     016      Cancer
     029      Clinical and Experimental Biochemistry
     005      General Pathology and Pathological Anatomy
LA   English
SL   English
ED   Entered STN: 28 Mar 2008
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Last Updated on STN: 28 Mar 2008

AB Background: There are several substances available to target members of the epidermal growth factor receptor (EGFR) family, both for imaging in nuclear medicine and for various forms of therapy. The level and stability of expression in both primary tumors and corresponding metastases is crucial in the assessment of a receptor as a target in systemic tumor therapy. To date, the expression of EGFR family members has only been determined in primary laryngeal carcinomas, and we have not found published data regarding the receptor status in corresponding metastatic lesions. Methods: Expression of EGFR, ***HER2*** , and HER3 was investigated immunohistochemically in both lymph node metastases and corresponding primary laryngeal squamous carcinomas (n = 40). Results: EGFR overexpression (2+ or 3+) was found in 87.5% (35/40) of the laryngeal primary tumors and 82.5% (33/40) of the corresponding lymph node metastases. There was a good agreement between the primary tumors and the paired metastases regarding EGFR expression. ***HER2*** overexpression was found in only four cases (10.5%) of the studied primary tumors and in all cases the ***HER2*** expression was retained in the paired metastases. Another two metastases gained ***HER2*** status when compared to the corresponding primary tumors. Strong HER3 staining was found in 26.7% of both the primary tumors and the corresponding metastases. Conclusions: The high frequency and stability in EGFR expression is encouraging for efforts to use EGFR targeting agents (e.g. Iressa, Tarceva, Erbitux or radiolabeled antibodies) for therapy of laryngeal carcinoma. For a few laryngeal carcinoma patients with ***HER2*** overexpression, anti- ***HER2*** agents could possibly be used. .COPYRGT. 2007 Society of Surgical Oncology.

L2 ANSWER 2 OF 16 EMBASE COPYRIGHT (c) 2009 Elsevier B.V. All rights reserved on STN DUPLICATE 2

AN 2008241208 EMBASE <<LOGINID::20090428>>

TI Differences in radiosensitivity between three ***HER2*** overexpressing cell lines.

AU Steffen, Ann-Charlott; Tolmachev, Vladimir; Stenerlow, Bo; ***Carlsson,*** *** Jorgen (correspondence)***

CS Unit of Biomedical Radiation Sciences, Department of Oncology, Radiology and Clinical Immunology, Uppsala University, Uppsala 751 85, Sweden. Jorgen.Carlsson@bms.uu.se

AU Gostring, Lovisa

CS Affibody AB, Bromma 161 02, Sweden.

AU Palm, Stig

CS Department of Radiation Physics, Sahlgrenska Academy, Goteborg University, Goteborg 413 45, Sweden.

AU ***Carlsson, Jorgen (correspondence)***

CS Biomedical Radiation Sciences, Rudbeck Laboratory, Uppsala 751 85, Sweden. Jorgen.Carlsson@bms.uu.se

SO European Journal of Nuclear Medicine and Molecular Imaging, (Jun 2008) Vol. 35, No. 6, pp. 1179-1191.
Refs: 40
ISSN: 1619-7070 CODEN: EJNMA6

CY Germany

DT Journal; Article

FS 023 Nuclear Medicine
029 Clinical and Experimental Biochemistry

LA English

SL English

ED Entered STN: 25 Jun 2008
Last Updated on STN: 25 Jun 2008
AB Purpose: ***HER2*** is a potential target for radionuclide therapy, especially when ***HER2*** overexpressing breast cancer cells are resistant to Herceptin.RTM. treatment. Therefore, it is of interest to analyse whether ***HER2*** overexpressing tumour cells have different inherent radiosensitivity. Methods: The radiosensitivity of three often used ***HER2*** overexpressing cell lines, SKOV-3, SKBR-3 and BT-474, was analysed. The cells were exposed to conventional photon irradiation, low linear energy transfer (LET), to characterise their inherent radiosensitivity. The analysis was made with clonogenic survival and growth extrapolation assays. The cells were also exposed to alpha particles, high LET, from (211)At decays using the ***HER2*** -binding affibody molecule (211)At-(Z(***HER2*** :4))(2) as targeting agent. Assays for studies of internalisation of the affibody molecule were applied. Results: SKOV-3 cells were most radioresistant, SKBR-3 cells were intermediate and BT-474 cells were most sensitive as measured with the clonogenic and growth extrapolation assays after photon irradiation. The ***HER2*** dependent cellular uptake of (211)At was qualitatively similar for all three cell lines. However, the sensitivity to the alpha particles from (211)At differed; SKOV-3 was most resistant, SKBR-3 intermediate and BT-474 most sensitive. These differences were unexpected because it is assumed that all types of cells should have similar sensitivity to high-LET radiation. The sensitivity to alpha particle exposure correlated with internalisation of the affibody molecule and with size of the cell nucleus. Conclusion: There can be differences in radiosensitivity, which, if they also exist between patient breast cancer cells, are important to consider for both conventional radiotherapy and for ***HER2*** -targeted radionuclide therapy. .COPYRGT. 2008 Springer-Verlag.

L2 ANSWER 3 OF 16 BIOSIS COPYRIGHT (c) 2009 The Thomson Corporation on STN DUPLICATE 3
AN 2009:37285 BIOSIS <<LOGINID::20090428>>
DN PREV200900037285
TI Dimeric ***HER2*** -specific affibody molecules inhibit proliferation of the SKBR-3 breast cancer cell line.
AU Ekerljung, Lina [Reprint Author]; Lindborg, Malin; Gedda, Lars; Frejd, Fredrik Y.; ***Carlsson, Jorgen*** ; Lennartsson, Johan
CS Uppsala Univ, Dept Oncol Radiol and Clin Immunol, Div Biomed Radiat Sci, Rudbeck Lab, SE-75185 Uppsala, Sweden
Lina.Ekerljung@bms.uu.se
SO Biochemical and Biophysical Research Communications, (DEC 12 2008) Vol. 377, No. 2, pp. 489-494.
CODEN: BBRCA9. ISSN: 0006-291X.
DT Article
LA English
ED Entered STN: 31 Dec 2008
Last Updated on STN: 31 Dec 2008
AB ***HER2*** -specific affibody molecules in different formats have previously been shown to be useful tumor targeting agents for radionuclide-based imaging and therapy applications, but their biological effect on tumor cells is not well known. In this study, two dimeric ((Z(***HER2*** :4))(2) and (Z(***HER2*** :342))(2)) and one monomeric (Z(***HER2*** :342)) ***HER2*** -specific affibody molecules are investigated with respect to biological activity. Both (Z(***HER2*** :4))(2) and (Z(***HER2*** :342))(2) were found to decrease the growth

rate of SKBR-3 cells to the same extent as the antibody trastuzumab. When the substances were removed, the cells treated with the dimeric affibody molecules continued to be growth suppressed while the cells treated with trastuzumab immediately resumed normal proliferation. The effects of Z(***HER2*** :342) were minor on both proliferation and cell signaling. The dimeric (Z(***HER2*** :4))(2) and (Z(***HER2*** :342))(2) both reduced growth of SKBR-3 cells and may prove therapeutically useful either by themselves or as carriers of radionuclides or other cytotoxic agents. (C) 2008 Elsevier Inc. All rights reserved.

L2 ANSWER 4 OF 16 BIOSIS COPYRIGHT (c) 2009 The Thomson Corporation on STN
DUPLICATE 4
AN 2007:286769 BIOSIS <<LOGINID::20090428>>
DN PREV200700282931
TI Radionuclide therapy of ***HER2*** -positive microxenografts using a Lu-177-labeled ***HER2*** -specific affibody molecule.
AU Tolmachev, Vladimir; Orlova, Anna; Pehrson, Rikard; Galli, Joakim; Baastrup, Barbro; Andersson, Karl; Sandstrom, Mattias; Rosik, Daniel; ***Carlsson, Jorgen*** ; Lundqvist, Hans; Wennborg, Anders; Nilsson, Fredrik Y. [Reprint Author]
CS Affibody AB, Box 20137, SE-16102 Bromma, Sweden
fredrik.nilsson@affibody.com
SO Cancer Research, (MAR 15 2007) Vol. 67, No. 6, pp. 2773-2782.
CODEN: CNREA8. ISSN: 0008-5472.
DT Article
LA English
ED Entered STN: 2 May 2007
Last Updated on STN: 2 May 2007
AB A radiolabeled anti- ***HER2*** Affibody molecule (Z(***HER2*** :342)) targets ***HER2*** -expressing xenografts with high selectivity and gives good imaging contrast. However, the small size (similar to 7 kDa) results in rapid glomerular filtration and high renal accumulation of radiometals, thus excluding targeted therapy. Here, we report that reversible binding to albumin efficiently reduces the renal excretion and uptake, enabling radio-metal-based nuclide therapy. The dimeric Affibody molecule (Z(***HER2*** :342))(2) Was fused with an albumin-binding domain (ABD) conjugated with the isothiocyanate derivative of CHX-A"-DTPA and labeled with the low-energy beta-emitter Lu-177. The obtained conjugate [CHX-A"-DTPA-ABD-(Z(***HER2*** :342))(2)] had a dissociation constant of IS pmol/L to ***HER2*** and 8.2 and 31 nmol/L for human and murine albumin, respectively. The radiolabeled conjugate displayed specific binding to ***HER2*** -expressing cells and good cellular retention in vitro. In vivo, fusion with ABD enabled a 25-fold reduction of renal uptake in comparison with the nonfused dimer molecule (Z(***HER2*** ,342))(2). Furthermore, the biodistribution showed high and specific uptake of the conjugate in ***HER2*** -expressing tumors. Treatment of SKOV-3 microxenografts (high ***HER2*** expression) with 17 or 22 MBq Lu-177-CHX-A"-DTPA-ABD-(Z(***HER2*** :342))(2) completely prevented formation of tumors, in contrast to mice given PBS or 22 MBq of a radiolabeled non- ***HER2*** -hinding Affibody molecule. In LS174T xenografts (low ***HER2*** expression), this treatment resulted in a small but significant increase of the survival time. Thus, fusion with ABD improved the in vivo biodistribution, and the results highlight Lu-177-CHX-A"-DTPA-ABD-(Z(***HER2*** :342))(2) as a candidate for treatment of disseminated tumors with a high level of ***HER2*** expression.

L2 ANSWER 5 OF 16 BIOSIS COPYRIGHT (c) 2009 The Thomson Corporation on STN
DUPLICATE 5
AN 2007:543629 BIOSIS <<LOGINID::20090428>>
DN PREV200700539165
TI EGFR, ***HER2*** and HER3 expression in esophageal primary tumours and corresponding metastases.
AU Wei, Qichun [Reprint Author]; Chen, Lirong; Sheng, Liming; Nordgren, Hans; Wester, Kenneth; ***Carlsson, Jorgen***
CS Zhejiang Univ, Sch Med, Inst Canc, Affiliated Hosp 2,Dept Radiat Oncol, Hangzhou 310009, Peoples R China
qichun.wei@bms.uu.se
SO International Journal of Oncology, (SEP 2007) Vol. 31, No. 3, pp. 493-499. ISSN: 1019-6439.
DT Article
LA English
ED Entered STN: 17 Oct 2007
Last Updated on STN: 17 Oct 2007
AB The expression of EGFR, ***HER2*** and HER3 receptors were analyzed in immunohistochemical preparations from primary esophageal tumours and corresponding lymph node metastases. The goal was to evaluate whether any of these receptors are suitable as targets for radionuclide based imaging and therapy. The receptor expressions were evaluated in parallel samples, primary tumour and metastasis, from each patient (n=51). The majority of the cases were esophageal squamous cell carcinomas, ESCC (n=40). The HercepTest scoring was used for the analysis of both ***HER2*** and EGFR expression (0, 1+, 2+ or 3+). HER3 was only evaluated as negative, weak or strong staining. EGFR overexpression (2+/3+) was found in 67.5% (27/40) of both the ESCC primary tumours and the corresponding lymph node metastases. There were only a few changes in these EGFR-scores: two cases from 2+/3+ to 0/1+ when the primary tumours were compared to the corresponding metastases and 2 changes the other way around. ***HER2*** overexpression (2+/3+) was found in only 3 of the primary ESCC tumours and 2 of the lymph node metastases. EGFR and ***HER2*** stainings were found mainly in the cell membranes. The HER3 staining (weak or strong) was mainly cytoplasmic and granular and was observed in about half (20/39) of the cases, for both the ESCC primary tumours and the corresponding lymph node metastases. It was concluded that ESCC lymph node metastases generally have a strong expression of EGFR in their cell membranes and to the same extent as in the primary tumours. The stability in EGFR expression is encouraging for efforts to develop radionuclide based EGFR imaging agents. It is also possible that EGFR targeting agents (e.g. Iressa, Tarceva, Erbitux or radiolabelled antibodies) can be applied for therapy of ESCC.

L2 ANSWER 6 OF 16 SCISEARCH COPYRIGHT (c) 2009 The Thomson Corporation on STN
AN 2007:103732 SCISEARCH <<LOGINID::20090428>>
GA The Genuine Article (R) Number: 123TX
TI [Lu-177]pertuzumab: Experimental therapy of HER-2-expressing xenografts
AU Persson, Mikael (Reprint)
CS Uppsala Univ, Rudbeck Lab, Dept Biomed Radiat Sci, SE-75185 Uppsala, Sweden (Reprint)
AU Gedda, Lars; Lundqvist, Hans; Tolmachev, Vladimir; Nordgren, Hans; Malmstrom, Per-Uno; ***Carlsson, Jorgen***
CS Uppsala Univ, Rudbeck Lab, Dept Expt Urol, SE-75185 Uppsala, Sweden; Uppsala Univ, Rudbeck Lab, Dept Mol & Morphol Pathol, SE-75185 Uppsala, Sweden

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CYA Sweden
SO CANCER RESEARCH, (1 JAN 2007) Vol. 67, No. 1, pp. 326-331.
ISSN: 0008-5472.
PB AMER ASSOC CANCER RESEARCH, 615 CHESTNUT ST, 17TH FLOOR, PHILADELPHIA, PA 19106-4404 USA.
DT Article; Journal
LA English
REC Reference Count: 22
ED Entered STN: 1 Feb 2007
Last Updated on STN: 1 Feb 2007
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
AB Pertuzumab (Omnitarg) is a novel antibody against HER-2, domain II. HER-2 is a tyrosine kinase receptor that is overexpressed in several carcinomas, especially breast cancer. Pertuzumab, labeled with the low-energy beta emitter Lu-177, might be a candidate for targeted radiotherapy of disseminated HER-2-positive micrometastases. The radiolabeled antibody [Lu-177]pertuzumab showed favorable targeting properties in BALB/c (nu/nu) mice with HER-2-overexpressing xenografts. The absorbed dose in tumors was more than five times higher than the absorbed dose in blood and more than seven times the absorbed dose in any other normal organ. Experimental therapy showed that [Lu-177]pertuzumab delayed tumor progression compared with controls (no treatment, $P < 0.0001$; nonlabeled pertuzumab antibody, $P < 0.0001$; and Lu-177-labeled irrelevant antibody, $P < 0.01$). No adverse side effects of the treatment could be detected. Thus, the experimental results support the planning of clinical studies applying [Lu-177]pertuzumab for therapy.

L2 ANSWER 7 OF 16 EMBASE COPYRIGHT (c) 2009 Elsevier B.V. All rights reserved on STN DUPLICATE 6
AN 2006208451 EMBASE <<LOGINID::20090428>>
TI Tumor imaging using a picomolar affinity ***HER2*** binding Affibody molecule.
AU Orlova, Anna; Magnusson, Mikaela; Eriksson, Tove L.J.; Nilsson, Martin; Larsson, Barbro; Hoiden-Guthenberg, Ingmarie; Tolmachev, Vladimir; Nilsson, Fredrik Y. (correspondence)
CS Affibody AB, Bromma, Sweden. fredrik.nilsson@affibody.se
AU Widstrom, Charles
CS Department of Hospital Physics, Uppsala University Hospital.
AU Orlova, Anna; ***Carlsson, Jorgen*** ; Tolmachev, Vladimir; Nilsson, Fredrik Y. (correspondence)
CS Department of Oncology, Radiology, and Clinical Immunology, Rudbeck Laboratory, Uppsala University, Uppsala, Sweden. fredrik.nilsson@affibody.se
AU Stahl, Stefan
CS Department of Biotechnology, AlbaNova University Center, Royal Institute of Technology, Stockholm, Sweden.
AU Nilsson, Fredrik Y. (correspondence)
CS Affibody AB, Box 20137, SE-161 02 Bromma, Sweden. fredrik.nilsson@affibody.se
SO Cancer Research, (15 Apr 2006) Vol. 66, No. 8, pp. 4339-4348.
Refs: 48
ISSN: 0008-5472 CODEN: CNREA8
CY United States
DT Journal; Article
FS 016 Cancer
023 Nuclear Medicine

029 Clinical and Experimental Biochemistry
LA English
SL English
ED Entered STN: 19 May 2006
Last Updated on STN: 19 May 2006
AB The detection of cell-bound proteins that are produced due to aberrant gene expression in malignant tumors can provide important diagnostic information influencing patient management. The use of small radiolabeled targeting proteins would enable high-contrast radionuclide imaging of cancers expressing such antigens if adequate binding affinity and specificity could be provided. Here, we describe a ***HER2*** -specific 6 kDa Affibody molecule (hereinafter denoted Affibody molecule) with 22 pmol/L affinity that can be used for the visualization of ***HER2*** expression in tumors in vivo using gamma camera. A library for affinity maturation was constructed by re-randomization of relevant positions identified after the alignment of first-generation variants of nanomolar affinity (50 nmol/L). One selected Affibody molecule, Z(***HER2*** :342) showed a >2,200-fold increase in affinity achieved through a single-library affinity maturation step. When radioiodinated, the affinity-matured Affibody molecule showed clear, high-contrast visualization of ***HER2*** -expressing xenografts in mice as early as 6 hours post-injection. The tumor uptake at 4 hours post-injection was improved 4-fold (due to increased affinity) with 9% of the injected dose per gram of tissue in the tumor. Affibody molecules represent a new class of affinity molecules that can provide small sized, high affinity cancer-specific ligands, which may be well suited for tumor imaging. .COPYRGT.2006 American Association for Cancer Research.

L2 ANSWER 8 OF 16 BIOSIS COPYRIGHT (c) 2009 The Thomson Corporation on STN
AN 2006:587101 BIOSIS <<LOGINID::20090428>>
DN PREV200600597727
TI Imaging and therapeutic targeting of ***HER2*** -positive tumors using Affibody molecules.
AU Nilsson, Fredrik Y. [Reprint Author]; Orlova, Anna; Tolmachev, Vladimir; Lundqvist, Hans; ***Carlsson, Jorgen*** ; Widstrom, Charles; Sandstrom, Matrias; Pehtson, Rikard; Stahl, Stefan; Wennborg, Anders; Wennborg, Anders; Feldwisch, Joachim
CS BMS, Uppsala, Sweden
SO Proceedings of the American Association for Cancer Research Annual Meeting, (APR 2006) Vol. 47, pp. 878.
Meeting Info.: 97th Annual Meeting of the American-Association-for-Cancer-Research (AACR). Washington, DC, USA. April 01 -05, 2006. Amer Assoc Canc Res.
ISSN: 0197-016X.
DT Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LA English
ED Entered STN: 8 Nov 2006
Last Updated on STN: 8 Nov 2006

L2 ANSWER 9 OF 16 EMBASE COPYRIGHT (c) 2009 Elsevier B.V. All rights reserved on STN DUPLICATE 7
AN 2006271370 EMBASE <<LOGINID::20090428>>
TI Affibody-mediated tumour targeting of HER-2 expressing xenografts in mice.
AU Steffen, Ann-Charlott (correspondence); Nilsson, Fredrik Y.; Tolmachev, Vladimir; ***Carlsson, Jorgen***
CS Department of Oncology, Radiology and Clinical Immunology, Rudbeck

Laboratory, Uppsala University, 751 85 Uppsala, Sweden. ann-charlott.steffen@bms.uu.se
AU Orlova, Anna; Nilsson, Fredrik Y.
CS Affibody AB, Bromma, Sweden.
AU Wikman, Maria; Stahl, Stefan
CS Department of Molecular Biotechnology, AlbaNova University Center, Royal Institute of Technology (KTH), Stockholm, Sweden.
AU Adams, Gregory P.
CS Department of Medical Oncology, Fox Chase Cancer Center, Philadelphia, PA, United States.
SO European Journal of Nuclear Medicine and Molecular Imaging, (Jun 2006) Vol. 33, No. 6, pp. 631-638.
Refs: 32
ISSN: 1619-7070 CODEN: EJNMA6
CY Germany
DT Journal; Article
FS 016 Cancer
023 Nuclear Medicine
030 Clinical and Experimental Pharmacology
037 Drug Literature Index
LA English
SL English
ED Entered STN: 21 Jun 2006
Last Updated on STN: 21 Jun 2006
AB Purpose: Targeted delivery of radionuclides for diagnostic and therapeutic applications has until recently largely been limited to receptor ligands, antibodies and antibody-derived molecules. Here, we present a new type of molecule, a 15-kDa bivalent affibody called (Z(***HER2*** :4))(2), with potential for such applications. The (Z(***HER2*** :4))(2) affibody showed high apparent affinity (K (D)=3 nM) towards the oncogene product HER-2 (also called p185/neu or c-erbB-2), which is often overexpressed in breast and ovarian cancers. The purpose of this study was to investigate the in vivo properties of the new targeting agent. Methods: The biodistribution and tumour uptake of the radioiodinated (Z(***HER2*** :4)) (2) affibody was studied in nude mice carrying tumours from xenografted HER-2 overexpressing SKOV-3 cells. Results: The radioiodinated (Z (***HER2*** :4))(2) affibody was primarily excreted through the kidneys, and significant amounts of radioactivity were specifically targeted to the tumours. The blood-borne radioactivity was, at all times, mainly in the macromolecular fraction. A tumour-to-blood ratio of about 10:1 was obtained 8 h post injection, and the tumours could be easily visualised with a gamma camera at this time point. Conclusion: The results indicate that the (Z (***HER2*** :4))(2) affibody is an interesting candidate for applications in nuclear medicine, such as radionuclide-based tumour imaging and therapy. .COPYRGT. Springer-Verlag 2006.

L2 ANSWER 10 OF 16 BIOSIS COPYRIGHT (c) 2009 The Thomson Corporation on STN DUPLICATE 8
AN 2008:7733 BIOSIS <<LOGINID::20090428>>
DN PREV200800009310
TI Comparative in vivo evaluation of technetium and iodine labels on an anti-***HER2*** Affibody for single-photon imaging of ***HER2*** expression in tumors.
AU Orlova, Anna; Nilsson, Fredrik Y.; Wikman, Maria; Widstrom, Charles; Stahl, Stefan; ***Carlsson, Jorgen*** ; Tolmachev, Vladimir [Reprint Author]

CS Uppsala Univ, Rudbeck Lab, Unit Biomed Radiat Sci, Dept Oncol Radiol and Clin Immunol, Uppsala 75185, Sweden
valdimir.tolmachev@bms.uu.se
SO Journal of Nuclear Medicine, (MAR 2006) Vol. 47, No. 3, pp. 512-519.
CODEN: JNMEAQ. ISSN: 0161-5505.
DT Article
LA English
ED Entered STN: 12 Dec 2007
Last Updated on STN: 12 Dec 2007
AB In vivo diagnosis with cancer-specific targeting agents that have optimal characteristics for imaging is an important development in treatment planning for cancer patients. Overexpression of the ***HER2*** antigen is high in several types of carcinomas and has predictive and prognostic value, especially for breast cancer. A new type of targeting agent, the Affibody molecule, was described recently. An Affibody dimer, HiS(6)-(ZHER(2:4))(2) (15.4 kDa), binds to ***HER2*** with an affinity of 3 nmol/L and might be used for the imaging of ***HER2*** expression. The use of Tc-99m might improve the availability of the labeled conjugate, and Tc(1)-carbonyl chemistry enables the site-specific labeling of the histidine tag on the Affibody molecule. The goals of the present study were to prepare Tc-99m-labeled HiOS(6)-(Z(***HER2*** :4))(2) and to evaluate its targeting properties compared with the targeting properties of I-125 -4-iodobenzoate-HiS(6)-(Z(***HER2*** :4))(2) [I-125-HiS(6)-(Z(***HER2*** :4))(2)]- Methods: The labeling of HiS6-(Z(***HER2*** :4))2 with Tc-99m was performed with an IsoLink kit. The specificity of Tc-99m-HiS(6)-(Z(***HER2*** :4))(2) binding to ***HER2*** was evaluated in vitro with SK-OV-3 ovarian carcinoma cells. The comparative biodistributions of Tc-99m-HiS(6)-(Z(***HER2*** ,4))(2) and I-125-HiS(6)-(Z(***HER2*** :4))(2) in tumor-bearing BALB/c nulnu mice were determined. Results: The labeling yield for Tc-99m-HIS6(Z(***HER2*** :4))(2) was similar to 60% (50 degrees C), and the radiochernical purity was greater than 97%. The conjugate was stable during storage and under histicline and cysteine challenges and demonstrated receptor-specific binding. The biodistribution study demonstrated tumor-specific uptake levels (percentage injected activity per gram of tissue [%]A/gj) of 2.6 %IA/g for Tc-99m-HiS(6)-(Z(***HER2*** :4))(2) and 2.3 % IA/g for I-125-HiS6-(Z(***HER2*** :4))(2) at 4 h after injection. Both conjugates provided clear imaging of SK-OV-3 xenografts at 6 h after injection. The tumor-to-nontumor ratios were much more favorable for the radioiodinated Affibody. Conclusion: The use of Tc(1)-carbonyl chemistry enabled us to prepare a stable, site-specifically labeled 99mTc-HiS(6)-(Z(***HER2*** :4))(2) conjugate that was able to bind to ***HER2*** -expressing cells in vitro and in vivo. The indirectly radioiodinated conjugate provided better tumor-to-liver ratios. The labeling of Affibody molecules with Tc-99m should be investigated further.

L2 ANSWER 11 OF 16 EMBASE COPYRIGHT (c) 2009 Elsevier B.V. All rights reserved on STN DUPLICATE 9
AN 2006372266 EMBASE <<LOGINID::20090428>>
TI Targeting the epidermal growth factor receptor family in radionuclide therapy of tumors-signal transduction and DNA repair.
AU Lennartsson, Johan (correspondence)
CS Ludwig Institute for Cancer Research, Uppsala University, Box 595, SE-751 24, Uppsala, Sweden. Johan.Lennartsson@LICR.uu.se
AU ***Carlsson, Jorgen*** ; Stenerlow, Bo
CS Department of Oncology, Radiology and Clinical Immunology, Rudbeck

Laboratory, Uppsala University, SE-751 85, Uppsala, Sweden.
SO Letters in Drug Design and Discovery, (2006) Vol. 3, No. 6, pp. 357-368.
Refs: 171
ISSN: 1570-1808
CY Netherlands
DT Journal; General Review; (Review)
FS 014 Radiology
016 Cancer
029 Clinical and Experimental Biochemistry
030 Clinical and Experimental Pharmacology
037 Drug Literature Index
LA English
SL English
ED Entered STN: 18 Aug 2006
Last Updated on STN: 18 Aug 2006
AB To therapeutically target disseminated tumor cells, while sparing the surrounding tissues, it is necessary to develop agents that interact with structures exposed selectively on the tumor cell surface. Members of the epidermal growth factor receptor family are commonly overexpressed in several tumor types and may serve as targeting structures. In this review we discuss the effects of EGFR and ***HER2*** targeting agents that can deliver radioactive nuclides, i.e. antibodies and affibody molecules, on intracellular signaling. If the targeting agent, in addition to deliver radioactivity to the tumor, can sensitize the tumor for its effects by influencing signal pathways that regulate cell survival and proliferation this will probably be advantageous. We discuss the changes in intracellular signaling that occurs after treatment of cancer cells with the clinically approved monoclonal antibodies cetuximab (anti-EGFR), trastuzumab (anti- ***HER2***) as well as ***HER2*** targeted affibody molecules which are under preclinical development. An important defence mechanism for cells against radiation is to activate DNA repair systems and we also address how DNA repair proteins are regulated in response to radiation or EGFR activation. .COPYRGT. 2006 Bentham Science Publishers Ltd.

L2 ANSWER 12 OF 16 EMBASE COPYRIGHT (c) 2009 Elsevier B.V. All rights reserved on STN DUPLICATE 10
AN 2006240306 EMBASE <<LOGINID::20090428>>
TI Effects of ***HER2*** -binding affibody molecules on intracellular signaling pathways.
AU Ekerljung, Lina; Steffen, Ann-Charlott; ***Carlsson, Jorgen***
CS Department of Oncology, Radiology and Clinical Immunology, Rudbeck Laboratory, Uppsala University, Uppsala, Sweden.
AU Lennartsson, Johan (correspondence)
CS Ludwig Institute for Cancer Research, Uppsala University, Uppsala, Sweden. Johan.Lennartsson@LICR.uu.se
AU Lennartsson, Johan (correspondence)
CS Ludwig Institute for Cancer Research, Uppsala University, Biomedical Center, SE-751 24 Uppsala, Sweden. Johan.Lennartsson@LICR.uu.se
SO Tumor Biology, (May 2006) Vol. 27, No. 4, pp. 201-210.
Refs: 28
ISSN: 1010-4283 CODEN: TUMBEA
CY Switzerland
DT Journal; Article
FS 037 Drug Literature Index
005 General Pathology and Pathological Anatomy
007 Pediatrics and Pediatric Surgery

LA English
SL English
ED Entered STN: 15 Jun 2006
Last Updated on STN: 15 Jun 2006
AB Background: ***HER2*** , which is overexpressed in 25-30% of human breast cancers, is a tyrosine kinase receptor critical for the signal transduction network that regulates proliferation, migration and apoptosis of cells. Method: We report the effects of two novel ***HER2*** -binding affibody molecules (Affibody.RTM.), (Z(***HER2*** :4))(2) and Z(***HER2*** :342), on intracellular signal transduction pathways (Erk1/2, Akt and PLC.gamma.1) using quantitative immunoblotting techniques and their biological effects in cell culture. The clinically approved antibody trastuzumab (Herceptin.RTM.) was used as reference substance. Results: Our data showed that, although all substances target ***HER2*** , the effects on the receptor and signaling molecules differed. For example, ***HER2*** phosphorylation was induced by trastuzumab and (Z(***HER2*** :4))(2) but inhibited by Z(***HER2*** :342). The effects these substances had on signal transduction correlated to some degree with changes in growth and migration, e.g. (Z(***HER2*** :4))(2) stimulated phosphorylation of Erk1/2 and PLC.gamma.1, as well as growth and migration, while Z (***HER2*** :342) did not. Z(***HER2*** :342) even inhibited phosphorylation of PLC.gamma.1 and migration. Conclusion: Our data suggest that Z (***HER2*** :342) is a promising small agent (7 kDa) that may be used as an alternative, or complement, to trastuzumab. If radiolabelled, it can hopefully also be used for ***HER2*** imaging and radionuclide therapy. Copyright .COPYRGT. 2006 S. Karger AG.

L2 ANSWER 13 OF 16 BIOSIS COPYRIGHT (c) 2009 The Thomson Corporation on STN DUPLICATE 11
AN 2005:246126 BIOSIS <<LOGINID::20090428>>
DN PREV200510026707
TI Analysis of ***HER2*** expression in primary urinary bladder carcinoma and corresponding metastases.
AU Gardmark, Truls [Reprint Author]; Wester, Kenneth; Torre, Manuel De La; ***Carlsson, Jorgen*** ; Malmstrom, Per-Uno
CS Uppsala Univ, Akad Hosp, Dept Surg Sci, Div Urol, SE-75185 Uppsala, Sweden Truls.Gardmark@surgsci.uu.se
SO BJU International, (MAY 2005) Vol. 95, No. 7, pp. 982-986.
ISSN: 1464-4096.
DT Article
LA English
ED Entered STN: 29 Jun 2005
Last Updated on STN: 29 Jun 2005
AB To evaluate the expression of ***HER2*** receptors (previously reported to be over-expressed in malignant urothelium) in both primary tumours and metastases of transitional cell cancer, using two different staining methods and two different scoring techniques, considering the potential use of these receptors as targets for planned systemic anti-***HER2*** nuclide-based treatment. ***HER2*** expression was evaluated with two different immunohistochemical methods in 90 patients with primary urinary bladder cancer tumours and corresponding metastases. Sections were first stained with the commercially available breast cancer test kit (HercepTest (R), Dako, Glostrup, Denmark). Parallel sections were then stained with a modified HercepTest procedure. Two different evaluation criteria were compared; the HercepTest score that requires >= 10% stained tumour cells (as for breast cancer) and a proposed 'Target score' that requires > 67% stained tumour cells. The latter score is

assumed to be preferable for ***HER2*** -targeted radionuclide therapy.Using the HercepTest kit, the Target score gave lower fractions of positive primary tumours and metastases than the HercepTest score. The modified HercepTest staining procedure and Target score gave high ***HER2*** values in 80% of primary tumours and 62% of metastases, which is considerably more than that obtained with the HercepTest staining and score. There was a significant decrease in ***HER2*** positivity with increasing distance from the primary tumour. In nine sentinel-node metastases assessed, all but one were ***HER2*** -positive. Considering all regional metastases, 74% were positive, and of distant metastases, 47%; 72% of the patients with positive primary tumours also expressed ***HER2*** in their metastases.When combining the modified HercepTest with customised evaluation criteria, more ***HER2*** -positive tumours were diagnosed. The degree of ***HER2*** down-regulation was significantly higher in distant than in regional metastases. ***HER2*** -targeted therapy may be an alternative or complementary to other methods in the future treatment of metastatic urinary bladder carcinoma.

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AN 2005363617 EMBASE <<LOGINID::20090428>>

TI Cellular uptake of radioiodine delivered by trastuzumab can be modified by the addition of epidermal growth factor.

AU Nordberg, Erika (correspondence); Steffen, Ann-Charlott; Persson, Mikael; Sundberg, Asa L.; ***Carlsson, Jorgen***

CS Division of Biomedical Radiation Sciences, Department of Oncology, Radiology and Clinical Immunology, Uppsala University, 751 85, Uppsala, Sweden. Erika.Nordberg@bms.uu.se

AU Persson, Mikael

CS Division of Experimental Urology, Department of Surgical Sciences, Uppsala University, Uppsala, Sweden.

AU Glimelius, Bengt

CS Division of Oncology, Department of Oncology, Radiology and Clinical Immunology, Uppsala University, Uppsala, Sweden.

SO European Journal of Nuclear Medicine and Molecular Imaging, (Jul 2005) Vol. 32, No. 7, pp. 771-777.
Refs: 39
ISSN: 1619-7070 CODEN: EJNMA6

CY Germany

DT Journal; Article

FS 016 Cancer
023 Nuclear Medicine
037 Drug Literature Index

LA English

SL English

ED Entered STN: 27 Oct 2005
Last Updated on STN: 27 Oct 2005

AB Purpose: The purpose of this study was to analyse whether non-radiolabelled epidermal growth factor (EGF) can modify the cellular uptake of (125)I when delivered as [(125)I]trastuzumab. (125)I was used as a marker for the diagnostically and therapeutically more interesting isotopes (123)I (SPECT), (124)I (PET) and (131)I (therapy). Methods: The cell-associated radioactivity was measured in squamous carcinoma A431 cells following addition of [(125)I]trastuzumab. Different concentrations of [(125)I]trastuzumab and unlabelled EGF were used, and the total,

membrane-bound and internalised radioactivity was measured. We also analysed how EGF and trastuzumab affected the cell growth. Results: It was generally found that the cellular (125)I uptake was decreased by the addition of EGF when [(125)I]trastuzumab was added for short incubation times. However, if the incubation times were longer, EGF increased the (125)I uptake. This shift came earlier when higher [(125)I]trastuzumab concentrations were applied. The addition of EGF also influenced cell proliferation, and concentrations above 10 ng/ml reduced cell growth by approximately 20% after 24 h of incubation. Conclusion: By adding unlabelled EGF, it was possible to modify the cellular uptake of [(125)I]trastuzumab. This points towards new approaches for the modification of radionuclide uptake in EGFR- and ***HER2*** -positive tumours. .COPYRGT. Springer-Verlag 2005.

L2 ANSWER 15 OF 16 BIOSIS COPYRIGHT (c) 2009 The Thomson Corporation on STN DUPLICATE 13

AN 2005:434799 BIOSIS <<LOGINID::20090428>>

DN PREV200510218038

TI In vitro characterization of a bivalent anti-HER-2 affibody with potential for radionuclide-based diagnostics.

AU Steffen, Ann-Charlott [Reprint Author]; Wikman, Maria; Tolmachev, Vladimir; Adams, Gregory P.; Nilsson, Fredrik Y.; Stahl, Stefan; ***Carlsson, Jorgen***

CS Uppsala Univ, Dept Oncol, Rudbeck Lab, Unit Biomed Radiat Sci, Hammarskolds Vag 20, S-75237 Uppsala, Sweden
ann-charlott.steffen@bms.uu.se

SO Cancer Biotherapy & Radiopharmaceuticals, (JUN 2005) Vol. 20, No. 3, pp. 239-248.
ISSN: 1084-9785.

DT Article

LA English

ED Entered STN: 26 Oct 2005
Last Updated on STN: 26 Oct 2005

AB The 185 kDa transmembrane glycoprotein human epidermal growth factor receptor 2 (HER-2) (p185/neu, c-ErbB-2) is overexpressed in breast and ovarian cancers. Overexpression in breast cancer correlates with poor patient prognosis, and visualization of HER-2 expression might provide valuable diagnostic information influencing patient management. We have previously described the generation of a new type of affinity ligand, a 58-amino-acid affibody (Z(***HER2*** :4)) with specific binding to HER-2. In order to benefit from avidity effects, we have created a bivalent form of the affibody ligand, (Z(***HER2*** :4))(2). The monovalent and bivalent ligands were compared in various assays. The new bivalent affibody has a molecular weight of 15.6 kDa and an apparent affinity (K-D) against HER-2 of 3 W After radioiodination, using the linker molecule N-succinimidyl p-(trimethylstannyl) benzoate (SPMB), in vitro binding assays showed specific binding to HER-2 overexpressing cells. Internalization of I-125 was shown after delivery with both the monovalent and the bivalent affibody. The cellular retention of I-125 was longer after delivery with the bivalent affibody when,compared to delivery with the monovalent affibody. With approximately the same affinity as the monoclonal antibody trastuzumab (Herceptin (TM)) but only one tenth of the size, this new bivalent molecule is a promising candidate for radionuclide-based detection of HER-2 expression in tumors. I-125 was used in this study as a surrogate marker for the diagnostically relevant radioisotopes I-123 for single photon emission computed tomography (SPECT)/gamma-camera imaging and I-124 for positron emission tomography

(PET).

L2 ANSWER 16 OF 16 BIOSIS COPYRIGHT (c) 2009 The Thomson Corporation on STN DUPLICATE 14
AN 2004:259300 BIOSIS <<LOGINID::20090428>>
DN PREV200400260223
TI Radiobromination of monoclonal antibody using potassium (76Br) (4 isothiocyanatobenzyl-ammonio)-bromo-decahydro-closo-dodecaborate (Bromo-DABI).
AU Bruskin, Alexander; Sivaev, Igor; Persson, Mikael; Lundqvist, Hans; ***Carlsson, Jorgen*** ; Sjoberg, Stefan; Tolmachev, Vladimir [Reprint Author]
CS Unit of Biomedical Radiation Sciences, Rudbecklaboratoriet, Uppsala University, S-751 85, Uppsala, Sweden
Vladimir.Tolmachev@bms.uu.se
SO Nuclear Medicine and Biology, (February 2004) Vol. 31, No. 2, pp. 205-211. print.
ISSN: 0969-8051.
DT Article
LA English
ED Entered STN: 19 May 2004
Last Updated on STN: 19 May 2004
AB The use of charged linkers in attaching radiohalogens to tumor-seeking biomolecules may improve intracellular retention of the radioactive label after internalization and degradation of targeting proteins. Derivatives of polyhedral boron clusters, such as closo-dodecaborate (2-) anion, might be possible charged linkers. In this study, a bifunctional derivative of closo-dodecaborate, (4-isothiocyanatobenzyl-ammonio)-undecahydro-closo-dodecaborate (DABI) was labeled with positron-emitting nuclide 76Br (T 1/2=16.2 h) and coupled to anti- ***HER2*** /neu humanized antibody Trastuzumab. The overall labeling yield at optimized conditions was 80.7+-0.6%. The label was proven to be stable in vitro in physiological and a set of denaturing conditions. The labeled antibody retained its capacity to bind to HER-2/neu antigen expressing cells. The results of the study demonstrated feasibility for using derivatives of closo-dodecaborate in indirect labeling of antibodies for radioimmunoPET.

=> e stahl stefan/au
E1 49 STAHL SONJA/AU
E2 1 STAHL SR JAMES P/AU
E3 339 --> STAHL STEFAN/AU
E4 3 STAHL STEFAN DR/AU
E5 7 STAHL STEFAN W/AU
E6 22 STAHL STEFANIE/AU
E7 1 STAHL STEFANIE K/AU
E8 1 STAHL STEFEN/AU
E9 1 STAHL STEHEN M/AU
E10 10 STAHL STEN/AU
E11 1 STAHL STEN R/AU
E12 3 STAHL STEPHAN/AU

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L3 55 ("STAHL STEFAN"/AU OR "STAHL STEFAN DR"/AU OR "STAHL STEFAN W"/AU) AND (HER2 OR SPA)

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L4 19 DUP REM L3 (36 DUPLICATES REMOVED)

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YOU HAVE REQUESTED DATA FROM 19 ANSWERS - CONTINUE? Y/(N):y

L4 ANSWER 1 OF 19 CAPLUS COPYRIGHT 2009 ACS on STN DUPLICATE 1
AN 2009:410584 CAPLUS <<LOGINID::20090428>>
TI Engineered affinity proteins for tumour-targeting applications
AU Friedman, Mikaela; ***Stahl, Stefan***
CS Division of Molecular Biotechnology, School of Biotechnology, AlbaNova University Center, Royal Institute of Technology (KTH), Stockholm, SE-106 91, Swed.
SO Biotechnology and Applied Biochemistry (2009), 53(1), 1-29
CODEN: BABIEC; ISSN: 0885-4513
PB Portland Press Ltd.
DT Journal
LA English
AB Targeting of tumor-assocd. antigens is an expanding treatment modality in clin. oncol. as an alternative to, or in combination with, conventional treatments, such as chemotherapy, external-radiation therapy and surgery. Targeting of antigens that are unique or more highly expressed in tumors than in normal tissues can be used to increase the specificity and reduce the cytotoxic effect on normal tissues. Several targeting agents have been studied for clin. use, where monoclonal antibodies have been the ones most widely used. More than 20 monoclonal antibodies are approved for therapy today and the largest field is oncol. Advances in genetic engineering and in vitro selection technol. has enabled the feasible high-throughput generation of monoclonal antibodies, antibody derivs. [e.g. scFvs, Fab mols., dAbs (single-domain antibodies), diabodies and minibodies] and more recently also non-Ig scaffold proteins. Several of these affinity proteins have been investigated for both in vivo diagnostics and therapy. Affinity proteins in tumor-targeted therapy can affect tumor progression by altering signal transduction or by delivering a payload of toxin, drug or radionuclide. The ErbB receptor family has been extensively studied as biomarkers in tumor targeting, primarily for therapy using monoclonal antibodies. Two receptors in the ErbB family, EGFR (epidermal growth factor receptor) and ***HER2*** (epidermal growth factor receptor 2), are overexpressed in various malignancies and assocd. with poor patient prognosis and are therefore interesting targets for solid tumors. In the present review, strategies are described for tumor targeting of solid tumors using affinity proteins to deliver radionuclides, either for mol. imaging or radiotherapy. Antibodies, antibody derivs. and non-Ig scaffold proteins are discussed with a certain focus on the affibody (Affibody) mol.

L4 ANSWER 2 OF 19 BIOSIS COPYRIGHT (c) 2009 The Thomson Corporation on STN DUPLICATE 2
AN 2008:337162 BIOSIS <<LOGINID::20090428>>
DN PREV200800337161
TI Directed evolution to low nanomolar affinity of a tumor-targeting epidermal growth factor receptor-binding affibody molecule.
AU Friedman, Mikaela; Orlova, Anna; Johansson, Eva; Eriksson, Tove L. J.; Hoiden-Guthenberg, Ingmarie; Tolmachev, Vladimir; Nilsson, Fredrik Y.; ***Stahl, Stefan*** [Reprint Author]
CS Kungl Tekniska Hogskolan KTH, AlbaNova Univ Ctr, Dept Mol Biotechnol, SE-10691 Stockholm, Sweden

stefans@biotech.kth.se
SO Journal of Molecular Biology, (MAR 7 2008) Vol. 376, No. 5, pp. 1388-1402. CODEN: JMOBAK. ISSN: 0022-2836.
DT Article
LA English
ED Entered STN: 5 Jun 2008
Last Updated on STN: 20 Aug 2008
AB The epidermal growth factor receptor 1 (EGFR) is overexpressed in various malignancies and is associated with a poor patient prognosis. A small, receptor-specific, high-affinity imaging agent would be a useful tool in diagnosing malignant tumors and in deciding upon treatment and assessing the response to treatment. We describe here the affinity maturation procedure for the generation of Affibody molecules binding with high affinity and specificity to EGFR. A library for affinity maturation was constructed by rerandomization of selected positions after the alignment of first-generation binding variants. New binders were selected with phage display technology, using a single oligonucleotide in a single-library effort, and the best second-generation binders had an approximately 30-fold improvement in affinity (K-d = 5-10 nM) for the soluble extracellular domain of EGFR in biospecific interaction analysis using Biacore. The dissociation equilibrium constant, Kd, was also determined for the Affibody with highest affinity using EGFR-expressing A431 cells in flow cytometric analysis (K-d = 2.8 nM). A retained high specificity for EGFR was verified by a dot blot assay showing staining only of EGFR proteins among a panel of serum proteins and other EGFR family member proteins (***HER2*** , HER3, and HER4). The EGFR-binding Affibody molecules were radiolabeled with indium-111, showing specific binding to EGFR-expressing A431 cells and successful targeting of the A431 tumor xenografts with 4-6% injected activity per gram accumulated in the tumor 4 h postinjection. (c) 2008 Elsevier Ltd. All rights reserved.

L4 ANSWER 3 OF 19 BIOSIS COPYRIGHT (c) 2009 The Thomson Corporation on STN DUPLICATE 3
AN 2009:33777 BIOSIS <<LOGINID::20090428>>
DN PREV200900033777
TI Epitope mapping of antibodies using bacterial surface display.
AU Rockberg, Johan; Lofblom, John; Hjelm, Barbara; Uhlen, Mathias [Reprint Author]; ***Stahl, Stefan***
CS Royal Inst Technol KTH, Dept Mol Biotechnol, AlbaNova Univ Ctr, Sch Biotechnol, SE-10691 Stockholm, Sweden
mathias@biotech.kth.se
SO Nature Methods, (DEC 2008) Vol. 5, No. 12, pp. 1039-1045. ISSN: 1548-7091.
DT Article
LA English
ED Entered STN: 24 Dec 2008
Last Updated on STN: 24 Dec 2008
AB We describe a method for mapping the epitopes recognized by antibodies, based on bacterial surface expression of antigen protein fragments followed by antibody-based flow-cytometric sorting. We analyzed the binding sites of both monoclonal and polyclonal antibodies directed to three human protein targets: (i) the human epidermal growth factor receptor 2 (***HER2***), (ii) ephrin-B3 and (iii) the transcription factor SATB2. All monoclonal antibodies bound a single epitope, whereas the polyclonal antibodies showed, in each case, a binding pattern with one to five separate epitopes. A comparison of polyclonal and monoclonal antibodies raised to the same antigen showed overlapping binding epitopes.

We also demonstrated that bacterial cells with displayed protein fragments can be used as affinity ligands to generate epitope-specific antibodies. Our approach shows a path forward for systematic validation of antibodies for epitope specificity and cross-reactivity on a whole-proteome level.

L4 ANSWER 4 OF 19 BIOSIS COPYRIGHT (c) 2009 The Thomson Corporation on STN DUPLICATE 4
AN 2008:74017 BIOSIS <<LOGINID::20090428>>
DN PREV200800073809
TI Simplified characterization through site-specific protease-mediated release of affinity proteins selected by staphylococcal display.
AU Kronqvist, Nina; Lofblom, John; Severa, Denise; ***Stahl, Stefan*** ; Wernerus, Henrik [Reprint Author]
CS AlbaNova Univ Ctr, Royal Inst Technol, Sch Biotechnol, Dept Mol Biotechnol, Roslagstullsbacken 16, SE-10691 Stockholm, Sweden henrik@biotech.kth.se
SO FEMS Microbiology Letters, (JAN 2008) Vol. 278, No. 1, pp. 128-136. CODEN: FMLED7. ISSN: 0378-1097.
DT Article
LA English
ED Entered STN: 16 Jan 2008
Last Updated on STN: 16 Jan 2008
AB The production of candidate affinity proteins in a soluble form, for downstream characterization, is often a time-consuming step in combinatorial protein engineering methods. Here, a novel approach for efficient production of candidate clones is described based on direct cleavage of the affinity protein from the surface of Staphylococcus carnosus, followed by affinity purification. To find a suitable strategy, three new fusion protein constructs were created, introducing a protease site for specific cleavage and purification tags for affinity chromatography purifications into the staphylococcal display vector. The three modified strains were evaluated in terms of transformation frequency, surface expression level and protease cleavage efficiency. A protocol for efficient affinity purification of protease-released affinity proteins using the introduced fusion-tags was successfully used, and the functionality of protease-treated and purified proteins was verified in a biosensor assay. To evaluate the devised method, a previously selected ***HER2*** -specific affibody was produced applying the new principle and was used to analyze ***HER2*** expression on human breast cancer cells.

L4 ANSWER 5 OF 19 BIOSIS COPYRIGHT (c) 2009 The Thomson Corporation on STN DUPLICATE 5
AN 2008:49522 BIOSIS <<LOGINID::20090428>>
DN PREV200800043972
TI Affibody-mediated transferrin depletion for proteomics applications.
AU Gronwall, Caroline; Sjoberg, Anna; Ramstrom, Margareta; Hoidn-Guthenberg, Ingmarie; Hober, Sophia; Jonasson, Per; ***Stahl, Stefan*** [Reprint Author]
CS AlbaNova Univ Ctr, Sch Biotechnol, Royal Inst Technol, Dept Mol Biotechnol,KTH, SE-10691 Stockholm, Sweden stefan.stahl@biotech.kth.se
SO Biotechnology Journal, (NOV 2007) Vol. 2, No. 11, pp. 1389-1398. ISSN: 1860-6768.
DT Article
LA English

ED Entered STN: 4 Jan 2008
Last Updated on STN: 4 Jan 2008
AB An Affibody(R) (Affibody) ligand with specific binding to human transferrin was selected by phage display technology from a combinatorial protein library based on the staphylococcal protein A (***SpA***)-derived Z domain. Strong and selective binding of the selected Affibody ligand to transferrin was demonstrated using biosensor technology and dot blot analysis. Impressive specificity was demonstrated as transferrin was the only protein recovered by affinity chromatography from human plasma. Efficient Affibody-mediated capture of transferrin, combined with IgG- and HSA- depletion, was demonstrated for human plasma and cerebrospinal fluid (CSF). For plasma, 85% of the total transfierrin content in the samples was depleted after only two cycles of transferrin removal, and for CSF, 78% efficiency was obtained in single-step depletion. These results clearly suggest a potential for the development of Affibody-based resins for the removal of abundant proteins in proteomics analyses.

L4 ANSWER 6 OF 19 BIOSIS COPYRIGHT (c) 2009 The Thomson Corporation on STN DUPLICATE 6
AN 2006:397535 BIOSIS <<LOGINID::20090428>>
DN PREV200600389717
TI Tumor Imaging using a picomolar affinity ***HER2*** binding affibody molecule.
AU Orlova, Anna; Magnusson, Mikaela; Eriksson, Tove L.J.; Nilsson, Martin; Larsson, Barbro; Holden-Guthenherg, Ingmarie; Widstroem, Charles; Carlsson, Joergen; Tolmachev, Vladimir; ***Stahl, Stefan*** ; Nilsson, Fredrik Y. [Reprint Author]
CS Affibody AB, Box 20137, SE-16102 Bromma, Sweden
fredriknilsson@affibody.se
SO Cancer Research, (APR 15 2006) Vol. 66, No. 8, pp. 4339-4348.
CODEN: CNREA8. ISSN: 0008-5472.
DT Article
LA English
ED Entered STN: 9 Aug 2006
Last Updated on STN: 9 Aug 2006
AB The detection of cell-bound proteins that are produced due to aberrant gene expression in malignant tumors can provide important diagnostic information influencing patient management. The use of small radiolabeled targeting proteins would enable high-contrast radionuclide imaging of cancers expressing such antigens if adequate binding affinity and specificity could he provided. Here, we describe a ***HER2*** -specific 6 kDa Affibody molecule (hereinafter denoted Affibody molecule) with 22 pmol/L affinity that can be used for the visualization of ***HER2*** expression in tumors in vivo using gamma camera. A library for affinity maturation was constructed by re-randomization of relevant positions identified after the alignment of first-generation variants of nanomolar affinity (50 nmol/L). One selected Affibody molecule, Z(***HER2*** :342) showed a > 2,200-fold increase in affinity achieved through a single-library affinity maturation step. When radioiodinated, the affinity-matured Affibody molecule showed clear, high-contrast visualization of ***HER2*** -expressing xenografts in mice as early as 6 hours post-injection. The tumor uptake at 4 hours post-injection was improved 4-fold (due to increased affinity) with 9% of the injected dose per gram of tissue in the tumor. Affibody molecules represent a new class of affinity molecules that can provide small sized, high affinity cancer-specific ligands, which may be well suited for tumor imaging.

L4 ANSWER 7 OF 19 BIOSIS COPYRIGHT (c) 2009 The Thomson Corporation on STN
AN 2006:587101 BIOSIS <<LOGINID::20090428>>
DN PREV200600597727
TI Imaging and therapeutic targeting of ***HER2*** -positive tumors using Affibody molecules.
AU Nilsson, Fredrik Y. [Reprint Author]; Orlova, Anna; Tolmachev, Vladimir; Lundqvist, Hans; Carlsson, Jorgen; Widstrom, Charles; Sandstrom, Matrias; Pehtson, Rikard; ***Stahl, Stefan*** ; Wennborg, Anders; Wennborg, Anders; Feldwisch, Joachim
CS BMS, Uppsala, Sweden
SO Proceedings of the American Association for Cancer Research Annual Meeting, (APR 2006) Vol. 47, pp. 878.
Meeting Info.: 97th Annual Meeting of the American-Association-for-Cancer-Research (AACR). Washington, DC, USA. April 01 -05, 2006. Amer Assoc Canc Res.
ISSN: 0197-016X.
DT Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LA English
ED Entered STN: 8 Nov 2006
Last Updated on STN: 8 Nov 2006

L4 ANSWER 8 OF 19 CAPLUS COPYRIGHT 2009 ACS on STN DUPLICATE 7
AN 2006:526929 CAPLUS <<LOGINID::20090428>>
DN 145:511264
TI Affibody-mediated tumour targeting of HER-2 expressing xenografts in mice
AU Steffen, Ann-Charlott; Orlova, Anna; Wikman, Maria; Nilsson, Fredrik Y.; ***Stahl, Stefan*** ; Adams, Gregory P.; Tolmachev, Vladimir; Carlsson, Joergen
CS Department of Oncology, Radiology and Clinical Immunology, Rudbeck Laboratory, Uppsala University, Uppsala, Swed.
SO European Journal of Nuclear Medicine and Molecular Imaging (2006), 33(6), 631-638
CODEN: EJNMA6; ISSN: 1619-7070
PB Springer
DT Journal
LA English
AB Targeted delivery of radionuclides for diagnostic and therapeutic applications has until recently largely been limited to receptor ligands, antibodies and antibody-derived mols. Here, the authors present a new type of mol., a 15-kDa bivalent affibody called (ZHER2:4)2, with potential for such applications. The (ZHER2:4)2 affibody showed high apparent affinity (KD = 3 nM) towards the oncogene product HER-2 (also called p185/neu or c-erbB-2), which is often overexpressed in breast and ovarian cancers. The purpose of this study was to investigate the in vivo properties of the new targeting agent. The biodistribution and tumor uptake of the radioiodinated (ZHER2:4)2 affibody was studied in nude mice carrying tumors from xenografted HER-2 overexpressing SKOV-3 cells. The radioiodinated (ZHER2:4)2 affibody was primarily excreted through the kidneys, and significant amts. of radioactivity were specifically targeted to the tumors. The blood-borne radioactivity was, at all times, mainly in the macromol. fraction. A tumor-to-blood ratio of about 10:1 was obtained 8 h post injection, and the tumors could be easily visualized with a gamma camera at this time point. The results indicate that the (ZHER2:4)2 affibody is an interesting candidate for applications in nuclear medicine, such as radionuclide-based tumor imaging and therapy.
RE.CNT 32 THERE ARE 32 CITED REFERENCES AVAILABLE FOR THIS RECORD

ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 9 OF 19 BIOSIS COPYRIGHT (c) 2009 The Thomson Corporation on STN
DUPLICATE 8
AN 2008:7733 BIOSIS <<LOGINID::20090428>>
DN PREV200800009310
TI Comparative in vivo evaluation of technetium and iodine labels on an anti-***HER2*** Affibody for single-photon imaging of ***HER2*** expression in tumors.
AU Orlova, Anna; Nilsson, Fredrik Y.; Wikman, Maria; Widstrom, Charles; ***Stahl, Stefan*** ; Carlsson, Jorgen; Tolmachev, Vladimir [Reprint Author]
CS Uppsala Univ, Rudbeck Lab, Unit Biomed Radiat Sci, Dept Oncol Radiol and Clin Immunol, Uppsala 75185, Sweden
valdimir.tolmachev@bms.uu.se
SO Journal of Nuclear Medicine, (MAR 2006) Vol. 47, No. 3, pp. 512-519.
CODEN: JNMEAQ. ISSN: 0161-5505.
DT Article
LA English
ED Entered STN: 12 Dec 2007
Last Updated on STN: 12 Dec 2007
AB In vivo diagnosis with cancer-specific targeting agents that have optimal characteristics for imaging is an important development in treatment planning for cancer patients. Overexpression of the ***HER2*** antigen is high in several types of carcinomas and has predictive and prognostic value, especially for breast cancer. A new type of targeting agent, the Affibody molecule, was described recently. An Affibody dimer, HiS(6)-(ZHER(2:4))(2) (15.4 kDa), binds to ***HER2*** with an affinity of 3 nmol/L and might be used for the imaging of ***HER2*** expression. The use of Tc-99m might improve the availability of the labeled conjugate, and Tc(1)-carbonyl chemistry enables the site-specific labeling of the histidine tag on the Affibody molecule. The goals of the present study were to prepare Tc-99m-labeled Hi0S(6)-(Z(***HER2*** :4))(2) and to evaluate its targeting properties compared with the targeting properties of I-125 -4-iodobenzoate-HiS(6)-(Z(***HER2*** :4))(2) [I-125-HiS(6)-(Z(***HER2*** :4))(2)]- Methods: The labeling of HiS6-(Z(***HER2*** :4))2 with Tc-99m was performed with an IsoLink kit. The specificity of Tc-99m-HiS(6)-(Z(***HER2*** :4))(2) binding to ***HER2*** was evaluated in vitro with SK-OV-3 ovarian carcinoma cells. The comparative biodistributions of Tc-99m-HiS(6)-(Z(***HER2*** ,4))(2) and I-125-HiS(6)-(Z(***HER2*** :4))(2) in tumor-bearing BALB/c nulnu mice were determined. Results: The labeling yield for Tc-99m-HIS6(Z(***HER2*** :4))(2) was similar to 60% (50 degrees C), and the radiochernical purity was greater than 97%. The conjugate was stable during storage and under histicline and cysteine challenges and demonstrated receptor-specific binding. The biodistribution study demonstrated tumor-specific uptake levels (percentage injected activity per gram of tissue [%]A/gj) of 2.6 %IA/g for Tc-99m-HiS(6)-(Z(***HER2*** :4))(2) and 2.3 % IA/g for I-125-HiS6-(Z(***HER2*** :4))(2) at 4 h after injection. Both conjugates provided clear imaging of SK-OV-3 xenografts at 6 h after injection. The tumor-to-nontumor ratios were much more favorable for the radioiodinated Affibody. Conclusion: The use of Tc(1)-carbonyl chemistry enabled us to prepare a stable, site-specifically labeled 99mTc-HiS(6)-(Z(***HER2*** :4))(2) conjugate that was able to bind to ***HER2*** -expressing cells in vitro and in vivo. The indirectly radioiodinated conjugate provided better tumor-to-liver ratios. The labeling of Affibody molecules with Tc-99m should be investigated

further.

L4 ANSWER 10 OF 19 CAPLUS COPYRIGHT 2009 ACS on STN
AN 2005:34770 CAPLUS <<LOGINID::20090428>>
DN 142:109117
TI Her-2 receptor-binding derivatives of Staphylococcal protein A for use in diagnosis and therapy of cancer
IN Carlsson, Joergen; ***Stahl, Stefan*** ; Eriksson, Tove; Gunneriusson, Elin; Nilsson, Fredrik
PA Affibody AB, Swed.
SO PCT Int. Appl., 116 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 2005003156	A1	20050113	WO 2004-SE1049	20040630
	W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW				
	RW: BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
	AU 2004253835	A1	20050113	AU 2004-253835	20040630
	AU 2004253835	B2	20090129		
	CA 2531238	A1	20050113	CA 2004-2531238	20040630
	EP 1641818	A1	20060405	EP 2004-749087	20040630
	EP 1641818	B1	20081203		
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, FI, RO, CY, TR, BG, CZ, EE, HU, PL, SK				
	CN 1816563	A	20060809	CN 2004-80019059	20040630
	JP 2007537700	T	20071227	JP 2006-518586	20040630
	AT 416190	T	20081215	AT 2004-749087	20040630
	IN 2005KN02544	A	20061013	IN 2005-KN2544	20051209
PRAI	SE 2003-1987	A	20030704		
	SE 2004-275	A	20040209		
	WO 2004-SE1049	W	20040630		

AB Substitution derivs. of the Z domain of Staphylococcal protein A (***SPA***) with a strong, specific, binding affinity for ***HER2*** are described for use in the diagnosis and treatment of ***her2*** -dependent cancers. A gene for the protein and l expression vectors and host cells for manuf. of the protein are also described. Also provided is the use of such a polypeptide as a medicament, and as a targeting agent for directing substances conjugated thereto to cells overexpressing ***HER2*** . The specificity of binding of the protein for the receptor allows its use in drug targeting with minimal side effects. Methods, and kits for performing the methods, are also provided, which methods and kits rely on the binding of the polypeptide to ***HER2*** . The proteins were identified in combinatorial libraries by panning. The protein manufd. in Escherichia coli bound to ***HER2*** -bearing SKBR-3 cells. The protein was well-tolerated by injection when given to nude mice

bearing SKOV-3 cell implants. The protein was accumulated rapidly in SKOV-3 cells.
RE.CNT 6 THERE ARE 6 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 11 OF 19 BIOSIS COPYRIGHT (c) 2009 The Thomson Corporation on STN DUPLICATE 9
AN 2005:434799 BIOSIS <<LOGINID::20090428>>
DN PREV200510218038
TI In vitro characterization of a bivalent anti-HER-2 affibody with potential for radionuclide-based diagnostics.
AU Steffen, Ann-Charlott [Reprint Author]; Wikman, Maria; Tolmachev, Vladimir; Adams, Gregory P.; Nilsson, Fredrik Y.; ***Stahl, Stefan*** ; Carlsson, Jorgen
CS Uppsala Univ, Dept Oncol, Rudbeck Lab, Unit Biomed Radiat Sci, Hammarskolds Vag 20, S-75237 Uppsala, Sweden
ann-charlott.steffen@bms.uu.se
SO Cancer Biotherapy & Radiopharmaceuticals, (JUN 2005) Vol. 20, No. 3, pp. 239-248.
ISSN: 1084-9785.
DT Article
LA English
ED Entered STN: 26 Oct 2005
Last Updated on STN: 26 Oct 2005
AB The 185 kDa transmembrane glycoprotein human epidermal growth factor receptor 2 (HER-2) (p185/neu, c-ErbB-2) is overexpressed in breast and ovarian cancers. Overexpression in breast cancer correlates with poor patient prognosis, and visualization of HER-2 expression might provide valuable diagnostic information influencing patient management. We have previously described the generation of a new type of affinity ligand, a 58-amino-acid affibody (Z(***HER2*** :4)) with specific binding to HER-2. In order to benefit from avidity effects, we have created a bivalent form of the affibody ligand, (Z(***HER2*** :4))(2). The monovalent and bivalent ligands were compared in various assays. The new bivalent affibody has a molecular weight of 15.6 kDa and an apparent affinity (K-D) against HER-2 of 3 W After radioiodination, using the linker molecule N-succinimidyl p-(trimethylstannyl) benzoate (SPMB), in vitro binding assays showed specific binding to HER-2 overexpressing cells. Internalization of I-125 was shown after delivery with both the monovalent and the bivalent affibody. The cellular retention of I-125 was longer after delivery with the bivalent affibody when,compared to delivery with the monovalent affibody. With approximately the same affinity as the monoclonal antibody trastuzumab (Herceptin (TM)) but only one tenth of the size, this new bivalent molecule is a promising candidate for radionuclide-based detection of HER-2 expression in tumors. I-125 was used in this study as a surrogate marker for the diagnostically relevant radioisotopes I-123 for single photon emission computed tomography (SPECT)/gamma-camera imaging and I-124 for positron emission tomography (PET).

L4 ANSWER 12 OF 19 BIOSIS COPYRIGHT (c) 2009 The Thomson Corporation on STN DUPLICATE 10
AN 2002:426944 BIOSIS <<LOGINID::20090428>>
DN PREV200200426944
TI Vector engineering to improve a staphylococcal surface display system.
AU Wernerus, Henrik; ***Stahl, Stefan*** [Reprint author]
CS Department of Biotechnology, SCFAB, Royal Institute of Technology (KTH),

SE-106 91, Stockholm, Sweden
stefans@biotech.kth.se
SO FEMS Microbiology Letters, (18 June, 2002) Vol. 212, No. 1, pp. 47-54. print.
CODEN: FMLED7. ISSN: 0378-1097.
DT Article
LA English
ED Entered STN: 7 Aug 2002
Last Updated on STN: 7 Aug 2002
AB A previously developed expression system for surface display of heterologous proteins on the surface of Staphylococcus carnosus employs the secretion signals from a Staphylococcus hyicus lipase and the cell wall anchoring part of Staphylococcus aureus protein A (***SpA***) to achieve surface display of expressed recombinant proteins. The system has been successfully used in various applications but the vector has not been considered genetically stable enough to allow protein library display applications, which would be of obvious interest. A new set of vectors, differing in size and devoid of a phage f1 origin of replication, were constructed and evaluated in terms of bacterial growth characteristics and vector stability. Furthermore, surface expression of a model surface protein was monitored by an enzymatic whole-cell assay and flow cytometry. The engineered expression vectors demonstrated dramatically improved stability and growth properties and two of the novel vectors demonstrated retained high surface density of the displayed model protein. The flow cytometry was found to be a powerful tool for observing the surface density of displayed heterologous proteins, and would thus be a rational strategy for monitoring the optimisation of any surface display system. The implications of these improved display vectors for future protein library applications are discussed.

L4 ANSWER 13 OF 19 BIOSIS COPYRIGHT (c) 2009 The Thomson Corporation on STN DUPLICATE 11
AN 2002:589385 BIOSIS <<LOGINID::20090428>>
DN PREV200200589385
TI A novel affinity gene fusion system allowing protein A-based recovery of non-immunoglobulin gene products.
AU Graslund, Susanne; Eklund, Malin; Falk, Ronny; Uhlen, Mathias; Nygren, Per-Ake; ***Stahl, Stefan*** [Reprint author]
CS Division of Molecular Biotechnology, Department of Biotechnology, Royal Institute of Technology (KTH), SCFAB, SE-10691, Stockholm, Sweden
stefans@biochem.kth.se
SO Journal of Biotechnology, (9 October, 2002) Vol. 99, No. 1, pp. 41-50. print.
CODEN: JBITD4. ISSN: 0168-1656.
DT Article
LA English
ED Entered STN: 13 Nov 2002
Last Updated on STN: 13 Nov 2002
AB An expression vector system has been developed, taking advantage of a novel, Staphylococcus aureus protein A (***SPA***)-binding affinity tag ZSPA-1, enabling straightforward affinity blotting procedures and efficient recovery by affinity purification of expressed gene products on readily available reagents and chromatography media. The 58 amino acid ***SPA*** -binding affinity tag ZSPA-1, was previously selected from a library constructed by combinatorial mutagenesis of a protein domain from ***SPA*** . An Escherichia coli expression vector for intracellular T7 promoter (PT7) driven production was constructed with an N-terminal dual

affinity tag, consisting of a hexahistidyl (His6) tag in frame with the ZSPA-1 tag, thus allowing alternative affinity recovery methods. To evaluate the system, five cDNA clones from a mouse testis cDNA library were expressed, and two alternative blotting procedures were developed for convenient screening of expression efficiencies. The five produced fusion proteins were recovered on both immobilized metal-ion affinity chromatography (IMAC) columns and on Protein A-based chromatography media, to allow comparative studies. It was found that the Protein A-based recovery resulted in the highest degree of purity, and furthermore, gene products that were produced as inclusion bodies could after denaturation be efficiently affinity purified on Protein A-Sepharose in the presence of 0.5 M guanidine hydrochloride. The convenience and robustness of the presented expression system should make it highly suitable for various high-throughput protein expression efforts.

L4 ANSWER 14 OF 19 BIOSIS COPYRIGHT (c) 2009 The Thomson Corporation on STN DUPLICATE 12
AN 2000:260413 BIOSIS <<LOGINID::20090428>>
DN PREV200000260413
TI Improved systems for hydrophobic tagging of recombinant immunogens for efficient iscom incorporation.
AU Andersson, Christin; Sandberg, Lena; Wernerus, Henrik; Johansson, Margaretha; Lovgren-Bengtsson, Karin; ***Stahl, Stefan*** [Reprint author]
CS Department of Biotechnology, Kungliga Tekniska Hogskolan, S-100 44, Stockholm, Sweden
SO Journal of Immunological Methods, (April 21, 2000) Vol. 238, No. 1-2, pp. 181-193. print.
CODEN: JIMMBG. ISSN: 0022-1759.
DT Article
LA English
ED Entered STN: 21 Jun 2000
Last Updated on STN: 5 Jan 2002
AB We have previously reported a strategy for production in Escherichia coli of recombinant immunogens fused to a hydrophobic tag to improve their capacity to associate with an adjuvant formulation (Andersson et al., J. Immunol. Methods 222 (1999) 171). Here, we describe a further development of the previous strategy and present significant improvements. In the novel system, the target immunogen is produced with an N-terminal affinity tag suitable for affinity purification, and a C-terminal hydrophobic tag, which should enable association through hydrophobic interactions of the immunogen with an adjuvant system, here being immunostimulating complexes (iscoms). Two different hydrophobic tags were evaluated: (i) a tag denoted M, derived from the membrane-spanning region of Staphylococcus aureus protein A (***SpA***), and (ii) a tag denoted MI consisting of the transmembrane region of hemagglutinin from influenza A virus. Furthermore, two alternative affinity tags were evaluated; the serum albumin-binding protein ABP, derived from streptococcal protein G, and the divalent IgG-binding ZZ-domains derived from ***SpA*** . A malaria peptide M5, derived from the central repeat region of the Plasmodium falciparum blood-stage antigen Pf155/RESA, served as model immunogen in this study. Four different fusion proteins, ABP-M5-M, ABP-M5-MI, ZZ-M5-M and ZZ-M5-MI, were thus produced, affinity purified and evaluated in iscom-incorporation experiments. All of the fusion proteins were found in the iscom fractions in analytical ultracentrifugation, indicating iscom incorporation. This was further supported by electron microscopy analysis showing that iscoms were formed. In addition, these

iscom preparations were demonstrated to induce M5-specific antibody responses upon immunisation of mice, confirming the successful incorporation into iscoms. The novel system for hydrophobic tagging of immunogens, with optional affinity and hydrophobic tags, gave expression levels that were increased ten to fifty-fold, as compared to the earlier reported system. We believe that the presented strategy would be a convenient way to achieve efficient adjuvant association for recombinant immunogens.

L4 ANSWER 15 OF 19 BIOSIS COPYRIGHT (c) 2009 The Thomson Corporation on STN DUPLICATE 13
AN 1999:468986 BIOSIS <<LOGINID::20090428>>
DN PREV199900468986
TI Staphylococcal surface display of immunoglobulin A (IgA)- and IgE-specific in vitro-selected binding proteins (affibodies) based on Staphylococcus aureus protein A.
AU Gunneriusson, Elin; Samuelson, Patrik; Ringdahl, Jenny; Gronlund, Hans; Nygren, Per-Ake; ***Stahl, Stefan*** [Reprint author]
CS Department of Biotechnology, Royal Institute of Technology (KTH), S-100 44, Stockholm, Sweden
SO Applied and Environmental Microbiology, (Sept., 1999) Vol. 65, No. 9, pp. 4134-4140. print.
CODEN: AEMIDF. ISSN: 0099-2240.
DT Article
LA English
ED Entered STN: 9 Nov 1999
Last Updated on STN: 9 Nov 1999
AB An expression system designed for cell surface display of hybrid proteins on Staphylococcus carnosus has been evaluated for the display of Staphylococcus aureus protein A (***SpA***) domains, normally binding to immunoglobulin G (IgG) Fc but here engineered by combinatorial protein chemistry to yield ***SpA*** domains, denoted affibodies, with new binding specificities. Such affibodies, with human IgA or IgE binding activity, have previously been selected from a phage library, based on an ***SpA*** domain. In this study, these affibodies have been genetically
introduced in monomeric or dimeric forms into chimeric proteins expressed on the surface of S. carnosus by using translocation signals from a Staphylococcus hyicus lipase construct together with surface-anchoring regions of ***SpA*** . The recombinant surface proteins, containing the IgA- or IgE-specific affibodies, were demonstrated to be expressed as full-length proteins, localized and properly exposed at the cell surface of S. carnosus. Furthermore, these chimeric receptors were found to be functional, since recombinant S. carnosus cells were shown to have gained IgA and IgE binding capacity, respectively. In addition, a positive effect in terms of IgA and IgE reactivity was observed when dimeric versions of the affibodies were present. Potential applications for recombinant bacteria with redirected binding specificity in their surface proteins are discussed.

L4 ANSWER 16 OF 19 BIOSIS COPYRIGHT (c) 2009 The Thomson Corporation on STN DUPLICATE 14
AN 1999:255885 BIOSIS <<LOGINID::20090428>>
DN PREV199900255885
TI An in vitro selected binding protein (affibody) shows conformation-dependent recognition of the respiratory syncytial virus (RSV) G protein.

AU Hansson, Marianne; Ringdahl, Jenny; Robert, Alain; Power, Ultan; Goetsch, Liliane; Nguyen, Thien Ngoc; Uhlen, Mathias; ***Stahl, Stefan*** ; Nygren, Per-Ake [Reprint author]
CS Department of Biotechnology, Royal Institute of Technology (KTH), S-100 44, Stockholm, Sweden
SO Immunotechnology (Shannon), (March, 1999) Vol. 4, No. 3-4, pp. 237-252. print.
ISSN: 1380-2933.
DT Article
LA English
ED Entered STN: 2 Jul 1999
Last Updated on STN: 2 Jul 1999
AB Using phage-display technology, a novel binding protein (Z-affibody) showing selective binding to the RSV (Long strain) G protein was selected from a combinatorial library of a small alpha-helical protein domain (Z), derived from staphylococcal protein A (***SPA***). Biopanning of the Z-library against a recombinant fusion protein comprising amino acids 130-230 of the G protein from RSV-subgroup A, resulted in the selection of a Z-affibody (ZRSV1) which showed G protein specific binding. Using biosensor technology, the affinity (KD) between ZRSV1 and the recombinant protein was determined to be in the micromolar range (10-6 M). Interestingly, the ZRSV1 affibody was demonstrated to also recognize the partially (54%) homologous G protein of RSV subgroup B with similar affinity. Using different recombinant RSV G protein derived fragments, the binding was found to be dependent on the presence of the cysteinyl residues proposed to be involved in the formation of an intramolecular disulfide-constrained loop structure, indicating a conformation-dependent binding. Results from epitope mapping studies, employing a panel of monoclonal antibodies directed to different RSV G protein subfragments, suggest that the ZRSV1 affibody binding site is located within the region of amino acids 164-186 of the G protein. This region contains a 13 amino acid residue sequence which is totally conserved between subgroups A and B of RSV and extends into the cystein loop region (amino acids 173-186). The potential use of the RSV G protein-specific ZRSV1 affibody in diagnostic and therapeutic applications is discussed.

L4 ANSWER 17 OF 19 BIOSIS COPYRIGHT (c) 2009 The Thomson Corporation on STN DUPLICATE 15
AN 1999:63791 BIOSIS <<LOGINID::20090428>>
DN PREV199900063791
TI General expression vectors for production of hydrophobically tagged immunogens for direct iscom incorporation.
AU Andersson, Christin; Sandberg, Lena; Murby, Maria; Sjolander, Anders; Lovgren-Bengtsson, Karin; ***Stahl, Stefan*** [Reprint author]
CS Dep. Biotechnology, Kungliga Tekniska Hogskolan, S-100 44 Stockholm, Sweden
SO Journal of Immunological Methods, (Jan. 1, 1999) Vol. 222, No. 1-2, pp. 171-182. print.
CODEN: JIMMBG. ISSN: 0022-1759.
DT Article
LA English
ED Entered STN: 16 Feb 1999
Last Updated on STN: 16 Feb 1999
AB A new general strategy for the production of recombinant protein immunogens has been investigated. The rationale involves the production of a recombinant immunogen as fused to a composite tag comprising one domain suitable for affinity purification and a hydrophobic tag designed

for direct incorporation through hydrophobic interaction of the affinity-purified immunogen into an adjuvant system, in this case immunostimulating complexes (iscoms). Three different hydrophobic tags were evaluated: (i) a tag denoted IW containing stretches of hydrophobic isoleucine (I) and tryptophan (W) residues; (ii) a tag denoted MI consisting of the transmembrane region of hemagglutinin from influenza A virus; and (iii) a tag denoted PD designed to be pH-dependent in such a way that an amphiphatic alpha-helix would be formed at low pH. As an affinity tag, an IgG-binding domain Z derived from Staphylococcus aureus protein A (***SpA***) was used, and a malaria peptide M5, derived from the central repeat region of the Plasmodium falciparum blood-stage antigen Pf155/RESA, served as a model immunogen in this study. Three different fusion proteins, IW-Z-M5, MI-Z-M5 and PD-Z-M5, were produced in Escherichia coli, and after affinity purification these were evaluated in iscom-incorporation experiments. Two of the fusion proteins, IW-Z-M5 and MI-Z-M5 were found in the iscom fraction following preparative ultracentrifugation, indicating iscom incorporation. This was further supported by electron microscopy analysis showing that iscoms were formed. Furthermore, these iscom preparations were demonstrated to induce efficient M5-specific antibody responses upon immunization of mice, confirming successful incorporation into iscoms. The implications of these results for the design and production of subunit vaccines are discussed.

L4 ANSWER 18 OF 19 CAPLUS COPYRIGHT 2009 ACS on STN
AN 1993:406796 CAPLUS <<LOGINID::20090428>>
DN 119:6796
OREF 119:1419a,1422a
TI Plasmodium falciparum: the immune response in rabbits to the clustered asparagine-rich protein (CARP) after immunization in Freund's adjuvant or immunostimulating complexes (ISCOMs)
AU Sjoelander, Anders; ***Stahl, Stefan*** ; Loevgren, Karin; Hansson, Marianne; Cavelier, Lucia; Walles, Astrid; Helmby, Helena; Wahlin, Birgitta; Morein, Bror; et al.
CS Dep. Immunol., Stockholm Univ., Stockholm, S-106 91, Swed.
SO Experimental Parasitology (1993), 76(2), 134-45
CODEN: EXPAAA; ISSN: 0014-4894
DT Journal
LA English
AB The P. falciparum clustered asparagine-rich protein (CARP) is a merozoite-assocd. antigen which contains approx. 30% asparagine. Anal. of the DNA sequences located 5' of the cloned 1.4-kb CARP gene in the P. falciparum genome suggests that this gene fragment may encode the complete CARP and that the gene product is a protein of mol. wt. (Mr) 50,000. To analyze the immunogenicity of CARP, the gene was expressed as a fusion protein with staphylococcal protein A (***SpA*** -CARP). Immunization of rabbits with ***SpA*** -CARP in Freund's complete adjuvant (FCA) resulted in a strong antibody response against CARP as measured in ELISA. This response was efficiently boosted and sustained over a long time while that induced by 2 immunizations with ***SpA*** -CARP in ISCOMs was weak and of shorter duration. In both instances, the antibody levels against CARP were further increased by a second booster injection consisting of either ***SpA*** -CARP or CARP fused to the serum albumin-binding region (BB) of streptococcal protein G (BB-CARP) in PBS, indicating that immunizations with ***SpA*** -CARP in FCA or ISCOMs had induced a CARP-specific immunol. memory. Boosting with BB-CARP in PBS was more efficient than boosting with ***SpA*** -CARP in PBS. In all rabbits,

the antibodies obtained after the booster with CARP in PBS were the most efficient inhibitors of merozoite invasion in vitro. The antisera reacted with the intracellular parasite in immunofluorescence and with a band of Mr 50,000 in immunoblotting while several high-mol.-wt. components as well as the one of Mr 50,000 were immunopptd. The specificity of the antibody responses varied between the different rabbits as indicated in ELISA, with short synthetic peptides representing different CARP sequences. Thus, a previously cloned genomic DNA fragment may encode the complete P. falciparum blood-stage antigen CARP and CARP is immunogenic in rabbits both when administered in FCA or ISCOMs.

L4 ANSWER 19 OF 19 LIFESCI COPYRIGHT 2009 CSA on STN
AN 2009:165219 LIFESCI <<LOGINID::20090428>>
TI Affibody-mediated transferrin depletion for proteomics applications
AU Gronwall, Caroline; Sjoberg, Anna; Ramstrom, Margareta; Hoiden-Guthenberg, Ingmarie; Hober, Sophia; Jonasson, Per; ***Stahl, Stefan***
CS Department of Molecular Biotechnology, School of Biotechnology, AlbaNova University Center, Royal Institute of Technology (KTH), Stockholm, Sweden; E-mail: stefan.stahliotech.kth.se
SO Biotechnology Journal [Biotechnol. J.], vol. 2, no. 11, pp. 1389-1398. ISSN: 1860-6768.
DT Journal
FS W; N3; J
LA English
SL English
AB An Affibody(r) (Affibody) ligand with specific binding to human transferrin was selected by phage display technology from a combinatorial protein library based on the staphylococcal protein A (***SpA***)-derived Z domain. Strong and selective binding of the selected Affibody ligand to transferrin was demonstrated using biosensor technology and dot blot analysis. Impressive specificity was demonstrated as transferrin was the only protein recovered by affinity chromatography from human plasma. Efficient Affibody-mediated capture of transferrin, combined with IgG- and HSA-depletion, was demonstrated for human plasma and cerebrospinal fluid (CSF). For plasma, 85% of the total transferrin content in the samples was depleted after only two cycles of transferrin removal, and for CSF, 78% efficiency was obtained in single-step depletion. These results clearly suggest a potential for the development of Affibody-based resins for the removal of abundant proteins in proteomics analyses.

=> e eriksson tove/au
E1 23 ERIKSSON TORNY/AU
E2 54 ERIKSSON TORSTEN/AU
E3 3 --> ERIKSSON TOVE/AU
E4 30 ERIKSSON TOVE L J/AU
E5 1 ERIKSSON TOVE L J DR/AU
E6 5 ERIKSSON TOVE LISA JENNY/AU
E7 3 ERIKSSON TRYGGVE/AU
E8 1 ERIKSSON TUA/AU
E9 825 ERIKSSON U/AU
E10 3 ERIKSSON U */AU
E11 3 ERIKSSON U B/AU
E12 5 ERIKSSON U DR/AU

=> s e3-e6 and (HER2 or SPA)
L5 11 ("ERIKSSON TOVE"/AU OR "ERIKSSON TOVE L J"/AU OR "ERIKSSON TOVE

L J DR"/AU OR "ERIKSSON TOVE LISA JENNY"/AU) AND (HER2 OR SPA)

=> dup rem l5
PROCESSING COMPLETED FOR L5
L6 3 DUP REM L5 (8 DUPLICATES REMOVED)

=> d 1-
YOU HAVE REQUESTED DATA FROM 3 ANSWERS - CONTINUE? Y/(N):y

L6 ANSWER 1 OF 3 BIOSIS COPYRIGHT (c) 2009 The Thomson Corporation on STN
DUPLICATE 1
AN 2008:337162 BIOSIS <<LOGINID::20090428>>
DN PREV200800337161
TI Directed evolution to low nanomolar affinity of a tumor-targeting epidermal growth factor receptor-binding affibody molecule.
AU Friedman, Mikaela; Orlova, Anna; Johansson, Eva; ***Eriksson, Tove L.*** ***** J.*** ; Hoiden-Guthenberg, Ingmarie; Tolmachev, Vladimir; Nilsson, Fredrik Y.; Stahl, Stefan [Reprint Author]
CS Kungl Tekniska Hogskolan KTH, AlbaNova Univ Ctr, Dept Mol Biotechnol, SE-10691 Stockholm, Sweden
stefans@biotech.kth.se
SO Journal of Molecular Biology, (MAR 7 2008) Vol. 376, No. 5, pp. 1388-1402.
CODEN: JMOBAK. ISSN: 0022-2836.
DT Article
LA English
ED Entered STN: 5 Jun 2008
Last Updated on STN: 20 Aug 2008

L6 ANSWER 2 OF 3 BIOSIS COPYRIGHT (c) 2009 The Thomson Corporation on STN
DUPLICATE 2
AN 2006:397535 BIOSIS <<LOGINID::20090428>>
DN PREV200600389717
TI Tumor Imaging using a picomolar affinity ***HER2*** binding affibody molecule.
AU Orlova, Anna; Magnusson, Mikaela; ***Eriksson, Tove L.J.*** ; Nilsson, Martin; Larsson, Barbro; Holden-Guthenberg, Ingmarie; Widstroem, Charles; Carlsson, Joergen; Tolmachev, Vladimir; Stahl, Stefan; Nilsson, Fredrik Y. [Reprint Author]
CS Affibody AB, Box 20137, SE-16102 Bromma, Sweden
fredriknilsson@affibody.se
SO Cancer Research, (APR 15 2006) Vol. 66, No. 8, pp. 4339-4348.
CODEN: CNREA8. ISSN: 0008-5472.
DT Article
LA English
ED Entered STN: 9 Aug 2006
Last Updated on STN: 9 Aug 2006

L6 ANSWER 3 OF 3 CAPLUS COPYRIGHT 2009 ACS on STN
AN 2005:34770 CAPLUS <<LOGINID::20090428>>
DN 142:109117
TI Her-2 receptor-binding derivatives of Staphylococcal protein A for use in diagnosis and therapy of cancer
IN Carlsson, Joergen; Stahl, Stefan; ***Eriksson, Tove*** ; Gunneriusson, Elin; Nilsson, Fredrik
PA Affibody AB, Swed.
SO PCT Int. Appl., 116 pp.

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     CODEN: PIXXD2
DT   Patent
LA   English
FAN.CNT 1
     PATENT NO.          KIND   DATE        APPLICATION NO.         DATE
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PI   WO 2005003156        A1    20050113    WO 2004-SE1049          20040630
         W:  AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH,
             CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD,
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             NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY,
             TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW
         RW: BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW, AM,
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             SN, TD, TG
     AU 2004253835         A1    20050113    AU 2004-253835          20040630
     AU 2004253835         B2    20090129
     CA 2531238            A1    20050113    CA 2004-2531238         20040630
     EP 1641818            A1    20060405    EP 2004-749087          20040630
     EP 1641818            B1    20081203
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     CN 1816563            A     20060809    CN 2004-80019059        20040630
     JP 2007537700         T     20071227    JP 2006-518586          20040630
     AT 416190             T     20081215    AT 2004-749087          20040630
     IN 2005KN02544        A     20061013    IN 2005-KN2544          20051209
PRAI SE 2003-1987          A     20030704
     SE 2004-275           A     20040209
     WO 2004-SE1049        W     20040630
RE.CNT  6      THERE ARE 6 CITED REFERENCES AVAILABLE FOR THIS RECORD
               ALL CITATIONS AVAILABLE IN THE RE FORMAT


=> e gunneriusson elin/au
E1            4      GUNNERHED MALIN/AU
E2           50      GUNNERIUSSON E/AU
E3           28 --> GUNNERIUSSON ELIN/AU
E4            3      GUNNERIUSSON EVA/AU
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E6           19      GUNNERIUSSON LARS/AU
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E12           1      GUNNERMANN DIETER/AU

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L7           14 ("GUNNERIUSSON E"/AU OR "GUNNERIUSSON ELIN"/AU) AND (HER2 OR
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=> dup rem l7
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L8            4 DUP REM L7 (10 DUPLICATES REMOVED)
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YOU HAVE REQUESTED DATA FROM 4 ANSWERS - CONTINUE? Y/(N):y

L8   ANSWER 1 OF 4  CAPLUS  COPYRIGHT 2009 ACS on STN
AN   2005:34770  CAPLUS <<LOGINID::20090428>>
DN   142:109117
TI   Her-2 receptor-binding derivatives of Staphylococcal protein A for use in
     diagnosis and therapy of cancer
IN   Carlsson, Joergen; Stahl, Stefan; Eriksson, Tove;   ***Gunneriusson,***
  ***     Elin***  ; Nilsson, Fredrik
PA   Affibody AB, Swed.
SO   PCT Int. Appl., 116 pp.
     CODEN: PIXXD2
DT   Patent
LA   English
FAN.CNT 1
     PATENT NO.          KIND   DATE        APPLICATION NO.        DATE
     --------------      ----   --------    -------------------    --------
PI   WO 2005003156        A1    20050113    WO 2004-SE1049         20040630
         W:  AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH,
             CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD,
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             LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI,
             NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY,
             TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW
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             EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE,
             SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE,
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     AU 2004253835         A1    20050113    AU 2004-253835         20040630
     AU 2004253835         B2    20090129
     CA 2531238            A1    20050113    CA 2004-2531238        20040630
     EP 1641818            A1    20060405    EP 2004-749087         20040630
     EP 1641818            B1    20081203
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     CN 1816563            A     20060809    CN 2004-80019059       20040630
     JP 2007537700         T     20071227    JP 2006-518586         20040630
     AT 416190             T     20081215    AT 2004-749087         20040630
     IN 2005KN02544        A     20061013    IN 2005-KN2544         20051209
PRAI SE 2003-1987          A     20030704
     SE 2004-275           A     20040209
     WO 2004-SE1049        W     20040630
RE.CNT  6     THERE ARE 6 CITED REFERENCES AVAILABLE FOR THIS RECORD
              ALL CITATIONS AVAILABLE IN THE RE FORMAT

L8   ANSWER 2 OF 4  CAPLUS  COPYRIGHT 2009 ACS on STN
AN   2005:14425  CAPLUS <<LOGINID::20090428>>
DN   142:108442
TI   Staphylococcal protein A (  ***SPA***  ) variants and use for separating
     insulin from biological samples using affinity chromatography
IN     ***Gunneriusson, Elin***  ; Feldwisch, Joachim; Nygren, Per-Ake
PA   Affibody AB, Swed.
SO   PCT Int. Appl., 79 pp.
     CODEN: PIXXD2
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DT Patent
LA English
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 2005000883	A1	20050106	WO 2004-SE1050	20040630

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW

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PRAI SE 2003-1936 A 20030630
RE.CNT 11 THERE ARE 11 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L8 ANSWER 3 OF 4 BIOSIS COPYRIGHT (c) 2009 The Thomson Corporation on STN
DUPLICATE 1
AN 2004:458754 BIOSIS <<LOGINID::20090428>>
DN PREV200400458361
TI Selection and characterization of ***HER2*** /neu-binding affibody ligands.
AU Wikman, M.; Steffen, A.-C.; ***Gunneriusson, E.*** ; Tolmachev, V.; Adams, G. P.; Carlsson, J.; Stahl, S. [Reprint Author]
CS AlbaNova Univ CtrDept Biotechnol, KTH, SE-10691, Stockholm, Sweden
stefans@biotech.kth.se
SO Protein Engineering Design & Selection, (May 2004) Vol. 17, No. 5, pp. 455-462. print.
ISSN: 1741-0126 (ISSN print).
DT Article
LA English
ED Entered STN: 24 Nov 2004
Last Updated on STN: 24 Nov 2004

L8 ANSWER 4 OF 4 BIOSIS COPYRIGHT (c) 2009 The Thomson Corporation on STN
DUPLICATE 2
AN 1999:468986 BIOSIS <<LOGINID::20090428>>
DN PREV199900468986
TI Staphylococcal surface display of immunoglobulin A (IgA)- and IgE-specific in vitro-selected binding proteins (affibodies) based on Staphylococcus aureus protein A.
AU ***Gunneriusson, Elin*** ; Samuelson, Patrik; Ringdahl, Jenny; Gronlund, Hans; Nygren, Per-Ake; Stahl, Stefan [Reprint author]
CS Department of Biotechnology, Royal Institute of Technology (KTH), S-100 44, Stockholm, Sweden
SO Applied and Environmental Microbiology, (Sept., 1999) Vol. 65, No. 9, pp. 4134-4140. print.
CODEN: AEMIDF. ISSN: 0099-2240.
DT Article
LA English
ED Entered STN: 9 Nov 1999
Last Updated on STN: 9 Nov 1999

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E2            1     NILSSON FREDRIE/AU
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E12           1     NILSSON G */AU

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                2 OR SPA)

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L10           13 DUP REM L9 (38 DUPLICATES REMOVED)

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L10  ANSWER 1 OF 13  BIOSIS  COPYRIGHT (c) 2009 The Thomson Corporation  on STN
     DUPLICATE 1
AN   2008:337162  BIOSIS <<LOGINID::20090428>>
DN   PREV200800337161
TI   Directed evolution to low nanomolar affinity of a tumor-targeting
     epidermal growth factor receptor-binding affibody molecule.
AU   Friedman, Mikaela; Orlova, Anna; Johansson, Eva; Eriksson, Tove L. J.;
     Hoiden-Guthenberg, Ingmarie; Tolmachev, Vladimir;   ***Nilsson, Fredrik***
  ***      Y.***  ; Stahl, Stefan [Reprint Author]
CS   Kungl Tekniska Hogskolan KTH, AlbaNova Univ Ctr, Dept Mol Biotechnol,
     SE-10691 Stockholm, Sweden
     stefans@biotech.kth.se
SO   Journal of Molecular Biology, (MAR 7 2008) Vol. 376, No. 5, pp. 1388-1402.
     CODEN: JMOBAK. ISSN: 0022-2836.
DT   Article
LA   English
ED   Entered STN: 5 Jun 2008
     Last Updated on STN: 20 Aug 2008
AB   The epidermal growth factor receptor 1 (EGFR) is overexpressed in various
     malignancies and is associated with a poor patient prognosis.  A small,
     receptor-specific, high-affinity imaging agent would be a useful tool in
     diagnosing malignant tumors and in deciding upon treatment and assessing
     the response to treatment.  We describe here the affinity maturation
     procedure for the generation of Affibody molecules binding with high
     affinity and specificity to EGFR.  A library for affinity maturation was
     constructed by rerandomization of selected positions after the alignment
     of first-generation binding variants.  New binders were selected with
     phage display technology, using a single oligonucleotide in a
     single-library effort, and the best second-generation binders had an
     approximately 30-fold improvement in affinity (K-d = 5-10 nM) for the
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soluble extracellular domain of EGFR in biospecific interaction analysis using Biacore. The dissociation equilibrium constant, Kd, was also determined for the Affibody with highest affinity using EGFR-expressing A431 cells in flow cytometric analysis (K-d = 2.8 nM). A retained high specificity for EGFR was verified by a dot blot assay showing staining only of EGFR proteins among a panel of serum proteins and other EGFR family member proteins (***HER2*** , HER3, and HER4). The EGFR-binding Affibody molecules were radiolabeled with indium-111, showing specific binding to EGFR-expressing A431 cells and successful targeting of the A431 tumor xenografts with 4-6% injected activity per gram accumulated in the tumor 4 h postinjection. (c) 2008 Elsevier Ltd. All rights reserved.

L10 ANSWER 2 OF 13 BIOSIS COPYRIGHT (c) 2009 The Thomson Corporation on STN
DUPLICATE 2
AN 2007:286769 BIOSIS <<LOGINID::20090428>>
DN PREV200700282931
TI Radionuclide therapy of ***HER2*** -positive microxenografts using a Lu-177-labeled ***HER2*** -specific affibody molecule.
AU Tolmachev, Vladimir; Orlova, Anna; Pehrson, Rikard; Galli, Joakim; Baastrup, Barbro; Andersson, Karl; Sandstrom, Mattias; Rosik, Daniel; Carlsson, Jorgen; Lundqvist, Hans; Wennborg, Anders; ***Nilsson, Fredrik***
*** Y.*** [Reprint Author]
CS Affibody AB, Box 20137, SE-16102 Bromma, Sweden
fredrik.nilsson@affibody.com
SO Cancer Research, (MAR 15 2007) Vol. 67, No. 6, pp. 2773-2782.
CODEN: CNREA8. ISSN: 0008-5472.
DT Article
LA English
ED Entered STN: 2 May 2007
Last Updated on STN: 2 May 2007
AB A radiolabeled anti- ***HER2*** Affibody molecule (Z(***HER2*** :342)) targets ***HER2*** -expressing xenografts with high selectivity and gives good imaging contrast. However, the small size (similar to 7 kDa) results in rapid glomerular filtration and high renal accumulation of radiometals, thus excluding targeted therapy. Here, we report that reversible binding to albumin efficiently reduces the renal excretion and uptake, enabling radio-metal-based nuclide therapy. The dimeric Affibody molecule (Z(***HER2*** :342))(2) Was fused with an albumin-binding domain (ABD) conjugated with the isothiocyanate derivative of CHX-A"-DTPA and labeled with the low-energy beta-emitter Lu-177. The obtained conjugate [CHX-A"-DTPA-ABD-(Z(***HER2*** :342))(2)] had a dissociation constant of IS pmol/L to ***HER2*** and 8.2 and 31 nmol/L for human and murine albumin, respectively. The radiolabeled conjugate displayed specific binding to ***HER2*** -expressing cells and good cellular retention in vitro. In vivo, fusion with ABD enabled a 25-fold reduction of renal uptake in comparison with the nonfused dimer molecule (Z(***HER2*** ,342))(2). Furthermore, the biodistribution showed high and specific uptake of the conjugate in ***HER2*** -expressing tumors. Treatment of SKOV-3 microxenografts (high ***HER2*** expression) with 17 or 22 MBq Lu-177-CHX-A"-DTPA-ABD-(Z(***HER2*** :342))(2) completely prevented formation of tumors, in contrast to mice given PBS or 22 MBq of a radiolabeled non- ***HER2*** -hinding Affibody molecule. In LS174T xenografts (low ***HER2*** expression), this treatment resulted in a small but significant increase of the survival time. Thus, fusion with ABD improved the in vivo biodistribution, and the results highlight Lu-177-CHX-A"-DTPA-ABD-(Z(***HER2*** :342))(2) as a candidate for

treatment of disseminated tumors with a high level of ***HER2*** expression.

L10 ANSWER 3 OF 13 BIOSIS COPYRIGHT (c) 2009 The Thomson Corporation on STN DUPLICATE 3
AN 2007:242720 BIOSIS <<LOGINID::20090428>>
DN PREV200700233657
TI Synthetic affibody molecules: A novel class of affinity ligands for molecular imaging of ***HER2*** -expressing malignant tumors.
AU Orlova, Anna; Tolmachev, Vladimir; Pehrson, Rikard; Lindborg, Malin; Tran, Thuy; Sandstrom, Mattias; ***Nilsson, Fredrik Y.*** ; Wennborg, Anders; Abrahmsen, Lars; Feldwisch, Joachim [Reprint Author]
CS Affibody AB, Voltavagen 13,POB 20137, SE-16102 Bromma, Sweden joachim.feldwisch@affibody.com
SO Cancer Research, (MAR 1 2007) Vol. 67, No. 5, pp. 2178-2186. CODEN: CNREA8. ISSN: 0008-5472.
DT Article
LA English
ED Entered STN: 11 Apr 2007
Last Updated on STN: 11 Jul 2007
AB The Affibody molecule Z(***HER2*** :342-pep2), site-specifically and homogeneously conjugated with a 1,4,7,10-tetra-azacylododecane-N,N', N",N '''-tetraacetic acid (DOTA) chelator, was produced in a single chemical process by peptide synthesis. DOTA-Z(***HER2*** :342-pep2) folds spontaneously and binds ***HER2*** with 65 pmol/L affinity. Efficient radiolabeling with > 95% incorporation of In-111 was achieved within 30 min at low (room temperature) and high temperatures (up to 90 degrees C). Tumor uptake of In-111-DOTA-Z(HER12:342-pep2) was specific for ***HER2*** -positive xenografts. A high tumor uptake of 23% injected activity per gram tissue, a tumor-to-blood ratio of > 7.5, and high-contrast gamma camera images were obtained already I h after injection. Pretreatment with Herceptin did not interfere with tumor targeting, whereas degradation of ***HER2*** using the heat shock protein 90 inhibitor 17-allylamino-geldanamycin before administration of In-111-DOTA-Z(***HER2*** :342-pep2) obliterated the tumor image. The present results show that radiolabeled synthetic DOTA-Z(***HER2*** :342-pep2) has the potential to become a clinically useful radiopharmaceutical for in vivo molecular imaging of ***HER2*** -expressing carcinomas.

L10 ANSWER 4 OF 13 CAPLUS COPYRIGHT 2009 ACS on STN DUPLICATE 4
AN 2007:328411 CAPLUS <<LOGINID::20090428>>
DN 147:85823
TI Affibody molecules: potential for in vivo imaging of molecular targets for cancer therapy
AU Tolmachev, Vladimir; Orlova, Anna; ***Nilsson, Fredrik Y.*** ; Feldwisch, Joachim; Wennborg, Anders; Abrahmsen, Lars
CS Affibody AB, Bromma, SE-161 02, Swed.
SO Expert Opinion on Biological Therapy (2007), 7(4), 555-568 CODEN: EOBTA2; ISSN: 1471-2598
PB Informa Healthcare
DT Journal; General Review
LA English
AB A review. Targeting radionuclide imaging of tumor-assocd. antigens may help to select patients who will benefit from a particular biol. therapy. Affibody mols. are a novel class of small (.apprx. 7 kDa) phage display-selected affinity proteins, based on the B-domain scaffold of

staphylococcal protein A. A large library (3 .times. 109 variants) has enabled selection of high-affinity (up to 22 pM) binders for a variety of tumor-assocd. antigens. The small size of Affibody mols. provides rapid tumor localization and fast clearance from nonspecific compartments. Preclin. studies have demonstrated the potential of Affibody mols. for specific and high-contrast radionuclide imaging of ***HER2*** in vivo, and pilot clin. data using indium-111 and gallium-68 labeled anti-***HER2*** Affibody tracer have confirmed its utility for radionuclide imaging in cancer patients.

RE.CNT 106 THERE ARE 106 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L10 ANSWER 5 OF 13 CAPLUS COPYRIGHT 2009 ACS on STN DUPLICATE 5
AN 2007:407442 CAPLUS <<LOGINID::20090428>>
DN 146:457571
TI Affibody molecules: new protein domains for molecular imaging and targeted tumor therapy
AU ***Nilsson, Fredrik Y.*** ; Tolmachev, Vladimir
CS Affibody AB, Bromma, SE-161 02, Swed.
SO Current Opinion in Drug Discovery & Development (2007), 10(2), 167-175
CODEN: CODDFF; ISSN: 1367-6733
PB Thomson Scientific
DT Journal; General Review
LA English
AB A review. Mol. imaging shows promise as a useful tool to aid drug discovery and development and also to provide important prognostic and predictive diagnostic information affecting patient management in the clinic. However, the use of mol. imaging diagnostically is not widely adopted, in part due to the lack of suitable targeting agents. Affibody mols. are a class of small and very stable protein domains, which can be used to selectively address a wide range of protein targets. Their small size enables high contrast radionuclide imaging and they can be produced by conventional peptide synthesis methods. Their potential utility in mol. imaging is highlighted in a large no. of animal studies using anti-***HER2*** Affibody tracers and has recently been validated in breast cancer patients with ***HER2*** -expressing metastases. The therapeutic efficacy of the Affibody mols. in this indication was demonstrated in preclin. models using a targeted radionuclide as the effector function. This review will focus on the recent use of Affibody mols. for mol. imaging and their application for radioimmunotherapy.

RE.CNT 65 THERE ARE 65 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L10 ANSWER 6 OF 13 BIOSIS COPYRIGHT (c) 2009 The Thomson Corporation on STN DUPLICATE 6
AN 2006:397535 BIOSIS <<LOGINID::20090428>>
DN PREV200600389717
TI Tumor Imaging using a picomolar affinity ***HER2*** binding affibody molecule.
AU Orlova, Anna; Magnusson, Mikaela; Eriksson, Tove L.J.; Nilsson, Martin; Larsson, Barbro; Holden-Guthenherg, Ingmarie; Widstroem, Charles; Carlsson, Joergen; Tolmachev, Vladimir; Stahl, Stefan; ***Nilsson,*** *** Fredrik Y.*** [Reprint Author]
CS Affibody AB, Box 20137, SE-16102 Bromma, Sweden
fredriknilsson@affibody.se
SO Cancer Research, (APR 15 2006) Vol. 66, No. 8, pp. 4339-4348.
CODEN: CNREA8. ISSN: 0008-5472.

DT Article
LA English
ED Entered STN: 9 Aug 2006
Last Updated on STN: 9 Aug 2006
AB The detection of cell-bound proteins that are produced due to aberrant gene expression in malignant tumors can provide important diagnostic information influencing patient management. The use of small radiolabeled targeting proteins would enable high-contrast radionuclide imaging of cancers expressing such antigens if adequate binding affinity and specificity could he provided. Here, we describe a ***HER2*** -specific 6 kDa Affibody molecule (hereinafter denoted Affibody molecule) with 22 pmol/L affinity that can be used for the visualization of ***HER2*** expression in tumors in vivo using gamma camera. A library for affinity maturation was constructed by re-randomization of relevant positions identified after the alignment of first-generation variants of nanomolar affinity (50 nmol/L). One selected Affibody molecule, Z(***HER2*** :342) showed a > 2,200-fold increase in affinity achieved through a single-library affinity maturation step. When radioiodinated, the affinity-matured Affibody molecule showed clear, high-contrast visualization of ***HER2*** -expressing xenografts in mice as early as 6 hours post-injection. The tumor uptake at 4 hours post-injection was improved 4-fold (due to increased affinity) with 9% of the injected dose per gram of tissue in the tumor. Affibody molecules represent a new class of affinity molecules that can provide small sized, high affinity cancer-specific ligands, which may be well suited for tumor imaging.

L10 ANSWER 7 OF 13 BIOSIS COPYRIGHT (c) 2009 The Thomson Corporation on STN
AN 2006:587101 BIOSIS <<LOGINID::20090428>>
DN PREV200600597727
TI Imaging and therapeutic targeting of ***HER2*** -positive tumors using Affibody molecules.
AU ***Nilsson, Fredrik Y.*** [Reprint Author]; Orlova, Anna; Tolmachev, Vladimir; Lundqvist, Hans; Carlsson, Jorgen; Widstrom, Charles; Sandstrom, Matrias; Pehtson, Rikard; Stahl, Stefan; Wennborg, Anders; Wennborg, Anders; Feldwisch, Joachim
CS BMS, Uppsala, Sweden
SO Proceedings of the American Association for Cancer Research Annual Meeting, (APR 2006) Vol. 47, pp. 878.
Meeting Info.: 97th Annual Meeting of the American-Association-for-Cancer-Research (AACR). Washington, DC, USA. April 01 -05, 2006. Amer Assoc Canc Res.
ISSN: 0197-016X.
DT Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LA English
ED Entered STN: 8 Nov 2006
Last Updated on STN: 8 Nov 2006

L10 ANSWER 8 OF 13 BIOSIS COPYRIGHT (c) 2009 The Thomson Corporation on STN
DUPLICATE 7
AN 2006:466033 BIOSIS <<LOGINID::20090428>>
DN PREV200600473509
TI In-111-benzyl-DTPA-Z(***HER2*** : 342), an affibody-based conjugate for in vivo imaging of ***HER2*** expression in malignant tumors.
AU Tolmachev, Vladimir [Reprint Author]; ***Nilsson, Fredrik Y.*** ; Widstrom, Charles; Andersson, Karl; Rosik, Daniel; Gedda, Lars; Wennborg, Anders; Orlova, Anna

CS Univ Uppsala, Rudbeck Lab, Div Biomed Radiat Sci, S-75185 Uppsala, Sweden
vladimir.tolmachev@bms.uu.se
SO Journal of Nuclear Medicine, (MAY 2006) Vol. 47, No. 5, pp. 846-853.
CODEN: JNMEAQ. ISSN: 0161-5505.
DT Article
LA English
ED Entered STN: 20 Sep 2006
Last Updated on STN: 20 Sep 2006
AB Data on expression of the ***HER2*** (erbB-2) receptor in breast carcinoma make it possible to select the most efficient treatment. There are strong indications that ***HER2*** expression possesses prognostic and predictive values in ovarian, prostate, and lung carcinomas as well. Visualization of ***HER2*** expression using radionuclide targeting can provide important diagnostic information. The Affibody Z(***HER2*** :342) is a short (similar to 7 kDa) phage-display-selected protein that binds ***HER2*** with an affinity of 22 pmol/L. The goal of this study was to evaluate whether In-111-labeled ***HER2*** : 342 can be used for imaging of ***HER2*** overexpression in vivo.Methods: Z(***HER2*** :342) was labeled with In-111 via isothiocyanate-benzyl-DTPA (DTPA is diethylenetriaminepentaacetic acid) and the conjugate was characterized in vitro and in vivo.Results: In-111-Benzyl-DTPA-Z(***HER2*** :342) preserved the capacity to bind living ***HER2*** -expressing cells specifically. The affinity of In-benzyl-DTPA-Z(***HER2*** :342) to ***HER2*** was 21 pmol/L according to surface plasmon resonance measurements. In nude mice bearing ***HER2*** -expressing SKOV-3 xenografts, a tumor uptake of 12% +/- 3% injected activity per gram and a tumor-to-blood ratio of about 100 were obtained 4 h after injection. Tumor uptake in vivo was receptor specific, as it could be blocked with an excess of nonlabeled Z(***HER2*** :342). ***HER2*** -expressing xeno-grafts were clearly imaged 4 h after injection using a gamma-camera.Conclusion: In-111-Benzyl-DTPA-Z(***HER2*** :342) is a promising candidate for visualization of ***HER2*** expression in carcinomas, using the single-photon detection technique.

L10 ANSWER 9 OF 13 CAPLUS COPYRIGHT 2009 ACS on STN DUPLICATE 8
AN 2006:526929 CAPLUS <<LOGINID::20090428>>
DN 145:511264
TI Affibody-mediated tumour targeting of HER-2 expressing xenografts in mice
AU Steffen, Ann-Charlott; Orlova, Anna; Wikman, Maria; ***Nilsson, Fredrik***
*** Y.*** ; Stahl, Stefan; Adams, Gregory P.; Tolmachev, Vladimir; Carlsson,
Joergen
CS Department of Oncology, Radiology and Clinical Immunology, Rudbeck Laboratory, Uppsala University, Uppsala, Swed.
SO European Journal of Nuclear Medicine and Molecular Imaging (2006), 33(6), 631-638
CODEN: EJNMA6; ISSN: 1619-7070
PB Springer
DT Journal
LA English
AB Targeted delivery of radionuclides for diagnostic and therapeutic applications has until recently largely been limited to receptor ligands, antibodies and antibody-derived mols. Here, the authors present a new type of mol., a 15-kDa bivalent affibody called (ZHER2:4)2, with potential for such applications. The (ZHER2:4)2 affibody showed high apparent

affinity (KD = 3 nM) towards the oncogene product HER-2 (also called p185/neu or c-erbB-2), which is often overexpressed in breast and ovarian cancers. The purpose of this study was to investigate the in vivo properties of the new targeting agent. The biodistribution and tumor uptake of the radioiodinated (ZHER2:4)2 affibody was studied in nude mice carrying tumors from xenografted HER-2 overexpressing SKOV-3 cells. The radioiodinated (ZHER2:4)2 affibody was primarily excreted through the kidneys, and significant amts. of radioactivity were specifically targeted to the tumors. The blood-borne radioactivity was, at all times, mainly in the macromol. fraction. A tumor-to-blood ratio of about 10:1 was obtained 8 h post injection, and the tumors could be easily visualized with a gamma camera at this time point. The results indicate that the (ZHER2:4)2 affibody is an interesting candidate for applications in nuclear medicine, such as radionuclide-based tumor imaging and therapy.

RE.CNT 32 THERE ARE 32 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L10 ANSWER 10 OF 13 BIOSIS COPYRIGHT (c) 2009 The Thomson Corporation on STN DUPLICATE 9

AN 2008:7733 BIOSIS <<LOGINID::20090428>>

DN PREV200800009310

TI Comparative in vivo evaluation of technetium and iodine labels on an anti-***HER2*** Affibody for single-photon imaging of ***HER2*** expression in tumors.

AU Orlova, Anna; ***Nilsson, Fredrik Y.*** ; Wikman, Maria; Widstrom, Charles; Stahl, Stefan; Carlsson, Jorgen; Tolmachev, Vladimir [Reprint Author]

CS Uppsala Univ, Rudbeck Lab, Unit Biomed Radiat Sci, Dept Oncol Radiol and Clin Immunol, Uppsala 75185, Sweden
valdimir.tolmachev@bms.uu.se

SO Journal of Nuclear Medicine, (MAR 2006) Vol. 47, No. 3, pp. 512-519.
CODEN: JNMEAQ. ISSN: 0161-5505.

DT Article

LA English

ED Entered STN: 12 Dec 2007
Last Updated on STN: 12 Dec 2007

AB In vivo diagnosis with cancer-specific targeting agents that have optimal characteristics for imaging is an important development in treatment planning for cancer patients. Overexpression of the ***HER2*** antigen is high in several types of carcinomas and has predictive and prognostic value, especially for breast cancer. A new type of targeting agent, the Affibody molecule, was described recently. An Affibody dimer, HiS(6)-(ZHER(2:4))(2) (15.4 kDa), binds to ***HER2*** with an affinity of 3 nmol/L and might be used for the imaging of ***HER2*** expression. The use of Tc-99m might improve the availability of the labeled conjugate, and Tc(1)-carbonyl chemistry enables the site-specific labeling of the histidine tag on the Affibody molecule. The goals of the present study were to prepare Tc-99m-labeled HiOS(6)-(Z(***HER2*** :4))(2) and to evaluate its targeting properties compared with the targeting properties of I-125 -4-iodobenzoate-HiS(6)-(Z(***HER2*** :4))(2) [I-125-HiS(6)-(Z(***HER2*** :4))(2)]- Methods: The labeling of HiS6-(Z(***HER2*** :4))2 with Tc-99m was performed with an IsoLink kit. The specificity of Tc-99m-HiS(6)-(Z(***HER2*** :4))(2) binding to ***HER2*** was evaluated in vitro with SK-OV-3 ovarian carcinoma cells. The comparative biodistributions of Tc-99m-HiS(6)-(Z(***HER2*** ,4))(2) and I-125-HiS(6)-(Z(***HER2*** :4))(2) in tumor-bearing BALB/c nulnu mice were determined. Results: The labeling yield for Tc-99m-HIS6(Z(

HER2 :4))(2) was similar to 60% (50 degrees C), and the radiochernical purity was greater than 97%. The conjugate was stable during storage and under histicline and cysteine challenges and demonstrated receptor-specific binding. The biodistribution study demonstrated tumor-specific uptake levels (percentage injected activity per gram of tissue [%]A/gj) of 2.6 %IA/g for Tc-99m-HiS(6)-(Z(***HER2*** :4))(2) and 2.3 % IA/g for I-125-HiS6-(Z(***HER2*** :4))(2) at 4 h after injection. Both conjugates provided clear imaging of SK-OV-3 xenografts at 6 h after injection. The tumor-to-nontumor ratios were much more favorable for the radioiodinated Affibody. Conclusion: The use of Tc(1)-carbonyl chemistry enabled us to prepare a stable, site-specifically labeled 99mTc-HiS(6)-(Z(***HER2*** :4))(2) conjugate that was able to bind to ***HER2*** -expressing cells in vitro and in vivo. The indirectly radioiodinated conjugate provided better tumor-to-liver ratios. The labeling of Affibody molecules with Tc-99m should be investigated further.

L10 ANSWER 11 OF 13 CAPLUS COPYRIGHT 2009 ACS on STN
AN 2005:34770 CAPLUS <<LOGINID::20090428>>
DN 142:109117
TI Her-2 receptor-binding derivatives of Staphylococcal protein A for use in diagnosis and therapy of cancer
IN Carlsson, Joergen; Stahl, Stefan; Eriksson, Tove; Gunneriusson, Elin; ***Nilsson, Fredrik***
PA Affibody AB, Swed.
SO PCT Int. Appl., 116 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 2005003156	A1	20050113	WO 2004-SE1049	20040630
	W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW				
	RW: BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
	AU 2004253835	A1	20050113	AU 2004-253835	20040630
	AU 2004253835	B2	20090129		
	CA 2531238	A1	20050113	CA 2004-2531238	20040630
	EP 1641818	A1	20060405	EP 2004-749087	20040630
	EP 1641818	B1	20081203		
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, FI, RO, CY, TR, BG, CZ, EE, HU, PL, SK				
	CN 1816563	A	20060809	CN 2004-80019059	20040630
	JP 2007537700	T	20071227	JP 2006-518586	20040630
	AT 416190	T	20081215	AT 2004-749087	20040630
	IN 2005KN02544	A	20061013	IN 2005-KN2544	20051209
PRAI	SE 2003-1987	A	20030704		
	SE 2004-275	A	20040209		

WO 2004-SE1049 W 20040630

AB Substitution derivs. of the Z domain of Staphylococcal protein A (***SPA***) with a strong, specific, binding affinity for ***HER2*** are described for use in the diagnosis and treatment of ***her2*** -dependent cancers. A gene for the protein and l expression vectors and host cells for manuf. of the protein are also described. Also provided is the use of such a polypeptide as a medicament, and as a targeting agent for directing substances conjugated thereto to cells overexpressing ***HER2*** . The specificity of binding of the protein for the receptor allows its use in drug targeting with minimal side effects. Methods, and kits for performing the methods, are also provided, which methods and kits rely on the binding of the polypeptide to ***HER2*** . The proteins were identified in combinatorial libraries by panning. The protein manufd. in Escherichia coli bound to ***HER2*** -bearing SKBR-3 cells. The protein was well-tolerated by injection when given to nude mice bearing SKOV-3 cell implants. The protein was accumulated rapidly in SKOV-3 cells.

RE.CNT 6 THERE ARE 6 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L10 ANSWER 12 OF 13 CAPLUS COPYRIGHT 2009 ACS on STN DUPLICATE 10

AN 2005:1145736 CAPLUS <<LOGINID::20090428>>

DN 144:47348

TI Evaluation of ((4-Hydroxyphenyl)ethyl)maleimide for Site-Specific Radiobromination of Anti- ***HER2*** Affibody

AU Mume, Eskender; Orlova, Anna; Larsson, Barbro; Nilsson, Ann-Sofie; ***Nilsson, Fredrik Y.*** ; Sjoeberg, Stefan; Tolmachev, Vladimir

CS Department of Chemistry, Organic Chemistry, Uppsala University, Uppsala, Swed.

SO Bioconjugate Chemistry (2005), 16(6), 1547-1555
CODEN: BCCHES; ISSN: 1043-1802

PB American Chemical Society

DT Journal

LA English

AB Affibody mols. are a new class of small phage-display selected proteins using a scaffold domain of the bacterial receptor protein A. They can be selected for specific binding to a large variety of protein targets. An affibody mol. binding with high affinity to a tumor antigen ***HER2*** was recently developed for radionuclide diagnostics and therapy in vivo. The use of the positron-emitting nuclide 76Br (T1/2 = 16.2 h) could improve the sensitivity of detection of ***HER2*** -expressing tumors. A site-specific radiobromination of a cysteine-contg. variant of the anti- ***HER2*** affibody, (ZHER2:4)2-Cys, using ((4-hydroxyphenyl)ethyl)maleimide (HPEM), was evaluated in this study. It was found that HPEM can be radiobrominated with an efficiency of 83 .+-. 0.4% and thereafter coupled to freshly reduced affibody with a yield of 65.3 .+-. 3.9%. A "one-pot" labeling enabled the radiochem. purity of the conjugate to exceed 97%. The label was stable against challenge with large excess of nonlabeled bromide and in a high molar strength soln. In vitro cell tests demonstrated that radiobrominated affibody binds specifically to the ***HER2*** -expressing cell-line, SK-OV-3. Biodistribution studies were performed in nude mice bearing SK-OV-3 xenografts.

RE.CNT 34 THERE ARE 34 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L10 ANSWER 13 OF 13 BIOSIS COPYRIGHT (c) 2009 The Thomson Corporation on

STN DUPLICATE 11

AN 2005:434799 BIOSIS <<LOGINID::20090428>>
DN PREV200510218038
TI In vitro characterization of a bivalent anti-HER-2 affibody with potential for radionuclide-based diagnostics.
AU Steffen, Ann-Charlott [Reprint Author]; Wikman, Maria; Tolmachev, Vladimir; Adams, Gregory P.; ***Nilsson, Fredrik Y.*** ; Stahl, Stefan; Carlsson, Jorgen
CS Uppsala Univ, Dept Oncol, Rudbeck Lab, Unit Biomed Radiat Sci, Hammarskolds Vag 20, S-75237 Uppsala, Sweden
ann-charlott.steffen@bms.uu.se
SO Cancer Biotherapy & Radiopharmaceuticals, (JUN 2005) Vol. 20, No. 3, pp. 239-248.
ISSN: 1084-9785.
DT Article
LA English
ED Entered STN: 26 Oct 2005
Last Updated on STN: 26 Oct 2005
AB The 185 kDa transmembrane glycoprotein human epidermal growth factor receptor 2 (HER-2) (p185/neu, c-ErbB-2) is overexpressed in breast and ovarian cancers. Overexpression in breast cancer correlates with poor patient prognosis, and visualization of HER-2 expression might provide valuable diagnostic information influencing patient management. We have previously described the generation of a new type of affinity ligand, a 58-amino-acid affibody (Z(***HER2*** :4)) with specific binding to HER-2. In order to benefit from avidity effects, we have created a bivalent form of the affibody ligand, (Z(***HER2*** :4))(2). The monovalent and bivalent ligands were compared in various assays. The new bivalent affibody has a molecular weight of 15.6 kDa and an apparent affinity (K-D) against HER-2 of 3 W After radioiodination, using the linker molecule N-succinimidyl p-(trimethylstannyl) benzoate (SPMB), in vitro binding assays showed specific binding to HER-2 overexpressing cells. Internalization of I-125 was shown after delivery with both the monovalent and the bivalent affibody. The cellular retention of I-125 was longer after delivery with the bivalent affibody when,compared to delivery with the monovalent affibody. With approximately the same affinity as the monoclonal antibody trastuzumab (Herceptin (TM)) but only one tenth of the size, this new bivalent molecule is a promising candidate for radionuclide-based detection of HER-2 expression in tumors. I-125 was used in this study as a surrogate marker for the diagnostically relevant radioisotopes I-123 for single photon emission computed tomography (SPECT)/gamma-camera imaging and I-124 for positron emission tomography (PET).

```
=> s ((HER2)or(human epidermal growth factor receptor 2))
L11      27846 ((HER2) OR(HUMAN EPIDERMAL GROWTH FACTOR RECEPTOR 2))

=> s ((SPA)or(staphylococcal protein a))
L12      33038 ((SPA) OR(STAPHYLOCOCCAL PROTEIN A))

=> s l11 and l12
L13         58 L11 AND L12

=> dup rem l13
PROCESSING COMPLETED FOR L13
L14          31 DUP REM L13 (27 DUPLICATES REMOVED)
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=> d bib ab kwic 1-
YOU HAVE REQUESTED DATA FROM 31 ANSWERS - CONTINUE? Y/(N):y

L14  ANSWER 1 OF 31  CAPLUS  COPYRIGHT 2009 ACS on STN
AN   2009:52947  CAPLUS <<LOGINID::20090428>>
DN   150:119817
TI   Prevention of disulfide bond reduction during recombinant production of
     polypeptides
IN   Kao, Yung-Hsiang; Schmidt, Melody Trexler; Laird, Michael W.; Wong, Rita
     L.; Hewitt, Daniel P.
PA   Genentech, Inc., USA
SO   PCT Int. Appl., 129pp.
     CODEN: PIXXD2
DT   Patent
LA   English
FAN.CNT 1
     PATENT NO.          KIND   DATE        APPLICATION NO.        DATE
     --------------      ----   --------    -------------------    --------
PI   WO 2009009523        A2    20090115    WO 2008-US69395        20080708
         W:  AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ,
             CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES,
             FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE,
             KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD,
             ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH,
             PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TJ,
             TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW
         RW: AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU,
             IE, IS, IT, LT, LU, LV, MC, MT, NL, NO, PL, PT, RO, SE, SI, SK,
             TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD,
             TG, BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW,
             AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
     US 20090053786         A1    20090226    US 2008-217745         20080708
PRAI US 2007-948677P        P     20070709
AB   The invention concerns methods and means for preventing the redn. of
     disulfide bonds during the recombinant prodn. of disulfide-contg.
     polypeptides.  In particular, the invention concerns the prevention of
     disulfide bond redn. during harvesting of disulfide-contg. polypeptides,
     including antibodies, from recombinant host cell cultures.
IT   Protein sequences
        (for heavy and light chain variable regions of humanized antibodies to
       CD20,   ***HER2***  , VEGF, and CD11a)
IT   Bone morphogenetic proteins
     CD19 (antigen)
     CD20 (antigen)
     CD3 (antigen)
     CD34 (antigen)
     CD4 (antigen)
     CD40 (antigen)
     CD8 (antigen)
     CTLA-4 (antigen)
     Growth factor receptors
     Hemopoietins
     Hormone receptors
     Insulin-like growth factor-binding proteins
     Integrins
     Interferons
```

Interleukins
Lipoproteins
Macrophage inflammatory protein 1
Platelet-derived growth factors
Protein D
RANTES (chemokine)
Rheumatoid factors
Staphylococcal ***protein*** ***A***
T cell receptors
Transforming growth factors
Tumor necrosis factors
neu (receptor)
RL: BMF (Bioindustrial manufacture); BSU (Biological study, unclassified); PUR (Purification or recovery); BIOL (Biological study); PREP (Preparation)
(prevention of disulfide bond redn. during recombinant prodn. of polypeptides)

L14 ANSWER 2 OF 31 CAPLUS COPYRIGHT 2009 ACS on STN DUPLICATE 1
AN 2009:170433 CAPLUS <<LOGINID::20090428>>
DN 150:299456
TI Re-targeted adenovirus vectors with dual specificity; binding specificities conferred by two different Affibody molecules in the fiber
AU Myhre, S.; Henning, P.; Friedman, M.; Stahl, S.; Lindholm, L.; Magnusson, M. K.
CS Got-A-Gene AB, Kullavik, Swed.
SO Gene Therapy (2009), 16(2), 252-261
CODEN: GETHEC; ISSN: 0969-7128
PB Nature Publishing Group
DT Journal
LA English
AB Vectors based on Adenovirus type 5 (Ad5) are among the most common vectors in cancer gene therapy trials to date. However, for increased efficiency and safety, Ad5 should be de-targeted from its native receptors and re-targeted to a tumor antigen. The authors have described earlier an Ad5 vector genetically re-targeted to the tumor antigen ***HER2*** /neu by a dimeric version of the Affibody mol. ZH inserted in the HI-loop of the fiber knob of a coxsackie and adenovirus receptor-binding ablated fiber. This virus showed almost wild-type growth characteristics and infected cells through ***HER2*** /neu. Here the authors generate vectors with double specificity by incorporating two different Affibody mols., ZH (***HER2*** /neu-binding) and ZT (Taq polymerase-binding), at different positions relative to one another in the HI-loop. Receptor-binding studies together with viral prodn. and gene transfer assays showed that the recombinant fiber with ZT in the first position and ZH in the second position (ZTZH) bound to both its targets, whereas surprisingly, the fiber with ZHZT was devoid of binding to ***HER2*** /neu. Hence, it is possible to construct a recombinant adenovirus with dual specificity after evaluating the best position for each ligand in the fiber knob.
RE.CNT 30 THERE ARE 30 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT
AB . . . and re-targeted to a tumor antigen. The authors have described earlier an Ad5 vector genetically re-targeted to the tumor antigen ***HER2*** /neu by a dimeric version of the Affibody mol. ZH inserted in the HI-loop of the fiber knob of a coxsackie and adenovirus receptor-binding ablated fiber. This virus showed almost wild-type growth characteristics and infected cells through ***HER2*** /neu. Here the

authors generate vectors with double specificity by incorporating two different Affibody mols., ZH (***HER2*** /neu-binding) and ZT (Taq polymerase-binding), at different positions relative to one another in the HI-loop. Receptor-binding studies together with viral prodn.. . . the second position (ZTZH) bound to both its targets, whereas surprisingly, the fiber with ZHZT was devoid of binding to ***HER2*** /neu. Hence, it is possible to construct a recombinant adenovirus with dual specificity after evaluating the best position for each ligand. . .

IT ***Staphylococcal*** ***protein*** ***A***
RL: BUU (Biological use, unclassified); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(Z-domain synthetic homologues; re-targeted adenovirus vectors with dual specificity using binding specificities conferred by two different Affibody mols. in fiber)

IT Chimeric gene
RL: BUU (Biological use, unclassified); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(fiber gene fusion with ***HER2*** /neu-binding Affibody gene and Taq polymerase-binding Affibody gene; re-targeted adenovirus vectors with dual specificity using binding specificities conferred by two different Affibody mols. in fiber)

IT Fusion proteins (chimeric proteins)
RL: BUU (Biological use, unclassified); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(fiber protein fusion with ***HER2*** /neu-binding Affibody protein and Taq polymerase-binding Affibody protein; re-targeted adenovirus vectors with dual specificity using binding specificities conferred by two different Affibody mols. in fiber)

L14 ANSWER 3 OF 31 CAPLUS COPYRIGHT 2009 ACS on STN
AN 2008:1448250 CAPLUS <<LOGINID::20090428>>
DN 150:19087
TI Single-chain Fc (ScFc) regions, binding polypeptides comprising same, and methods related thereto
IN Farrington, Grahma K.; Saeed-Kothe, Amna; Garber, Ellen; Lugovskoy, Alexey Alexandrovich
PA Biogen Idec MA Inc., USA
SO PCT Int. Appl., 230pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 2008143954	A2	20081127	WO 2008-US6260	20080514
WO 2008143954	A3	20090319		

W: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW

RW: AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, NO, PL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG, BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW,

AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AP, EA, EP, OA
PRAI US 2007-930227P P 20070514
AB The present invention features polypeptides comprising an Fc region comprising genetically-fused Fc moieties. In addn., the invention provides methods for treating or preventing a disease or disorder in subject by administering the binding polypeptides to diseased subject.
IT Antibodies and Immunoglobulins
RL: BSU (Biological study, unclassified); BIOL (Biological study) (***Her2*** ; prepn. of single-chain Fc Igs for therapy in cancer, infection or inflammation)
IT Complement
FcRn receptors
Gene, animal
Interleukin 1
Ligands
Peptides, biological studies
Staphylococcal ***protein*** ***A***
Streptococcal protein G
RL: BSU (Biological study, unclassified); BIOL (Biological study) (prepn. of single-chain Fc Igs for therapy in cancer, infection or inflammation)

L14 ANSWER 4 OF 31 CAPLUS COPYRIGHT 2009 ACS on STN
AN 2008:1248757 CAPLUS <<LOGINID::20090428>>
DN 149:478674
TI Poly(amino acid) targeting moieties
IN Alexis, Frank; Zhang, Liangfang; Radovic-Moreno, Aleksander F.; Gu, Frank X.; Basto, Pamela; Levy-Nissenbaum, Etgar; Chan, Juliana; Langer, Robert S.; Farokhzad, Omid C.
PA Massachusetts Institute of Technology, USA; The Brigham and Women's Hospital, Inc.
SO PCT Int. Appl., 130pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 2

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 2008124639	A2	20081016	WO 2008-US59491	20080404
	WO 2008124639	A3	20081127		
	W: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW			
	RW: AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, NO, PL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG, BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AP, EA, EP, OA			
	US 20090074828	A1	20090319	US 2008-98354	20080404
PRAI	US 2007-910097P	P	20070404		
	US 2007-938590P	P	20070517		
	US 2007-985104P	P	20071102		
	US 2007-986202P	P	20071107		

US 2007-990250P P 20071126
OS MARPAT 149:478674
AB The present invention generally relates to polymers and macromols., in particular, to polymers useful in particles such as nanoparticles. One aspect of the invention is directed to a method of developing nanoparticles with desired properties. In one set of embodiments, the method includes producing libraries of nanoparticles having highly controlled properties, which can be formed by mixing together two or more macromols. in different ratios. One or more of the macromols. may be a polymeric conjugate of a moiety to a biocompatible polymer. In some cases, the nanoparticle may contain a drug. Other aspects of the invention are directed to methods using nanoparticle libraries. For example, DSPE-PEG bioconjugate 0.03 mg was mixed with lecithin 0.07 mg in 2 mL aq. soln. contg. 4 % ethanol. Poly(D,L-lactic-co glycolic acid) (PLGA) 1 mg was dissolved in acetonitrile solvent 1 mL, to which 5 % docetaxel of the mass of PLGA was added. The lecithin/DSPE-PEG soln. was first heated up to 65.degree.C for 3 min. Then the PLGA soln. was added to the aq. soln. of lecithin/DSPE-PEG dropwise under gentle stirring. These mixts. were vortexed for 3 min, followed by stirring for 2 h. In order to remove all org. solvents, these mixts. were then dialyzed for another 3 h against PBS buffer.
IT Gene, animal
RL: THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(ERBB2, anti- ***HER2*** affibody; poly(amino acid) targeting moieties)
IT Acrylic polymers, biological studies
Bile acids
Collagens, biological studies
Diphosphonates
Estrogens
Fibrates
Hepatocyte growth factor
Lecithins
Monocyte chemoattractant protein-1
Peptides, biological studies
Polyamides, biological studies
Polyamines
Polyanhydrides
Polycarbonates, biological studies
Polyesters, biological studies
Polyethers, biological studies
Polymers, biological studies
Polyoxyalkylenes, biological studies
Polyoxymethylenes, biological studies
Polyphosphazenes
Polyureas
Polyurethanes, biological studies
Prostate-specific antigen
Proteins
Staphylococcal ***protein*** ***A***
Transforming growth factor .alpha.
Transforming growth factor .beta.
Tumor necrosis factors
RL: THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(poly(amino acid) targeting moieties)

L14 ANSWER 5 OF 31 CAPLUS COPYRIGHT 2009 ACS on STN

AN 2008:702941 CAPLUS <<LOGINID::20090428>>
DN 149:47381
TI Two helix segment derived from ***Staphylococcal*** ***protein*** ***A*** domain B comprising a pair of anti-parallel alpha helixes capable of binding a target
IN Syud, Faisal Ahmed; Webster, Jack M.
PA General Electric Company, USA
SO PCT Int. Appl., 42 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 2

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 2008070816	A2	20080612	WO 2007-US86708	20071207
	WO 2008070816	A3	20080918		
	W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW			
	RW: AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, PL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG, BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AP, EA, EP, OA			
	US 20080176278	A1	20080724	US 2006-608590	20061208
PRAI	US 2006-608590	A	20061208		

AB Provided herein are isolated polypeptides derived from the ***Staphylococcal*** ***protein*** ***A*** domain B comprising a pair of anti-parallel alpha helixes that are capable of binding a target. Also provided are nucleic acid sequences encoding such two helix binders, vectors contg. the nucleic acid sequences encoding for two helix binders, and host cells transformed with vectors contg. the nucleic acid sequences encoding for the two-helix binders. Also provided are methods of using the two helix binders. The polypeptides provided herein are derived from the Z-domain of protein A. The two helix binders provided herein demonstrate a binding affinity for the target in the range of about 50 pM to about 200 nM. The anti-IgG two helix binder described below in the Examples (SEQ ID NO.:7) has demonstrated an affinity of about 50 pM for its target, IgG. The anti- ***HER2*** two helix binder described in the Examples below (SEQ ID NO.:8) has demonstrated an affinity of about 150 nM for its target, ***HER2*** .

TI Two helix segment derived from ***Staphylococcal*** ***protein*** ***A*** domain B comprising a pair of anti-parallel alpha helixes capable of binding a target

AB Provided herein are isolated polypeptides derived from the ***Staphylococcal*** ***protein*** ***A*** domain B comprising a pair of anti-parallel alpha helixes that are capable of binding a target. Also provided are nucleic. . . below in the Examples (SEQ ID NO.:7) has demonstrated an affinity of about 50 pM for its target, IgG. The anti- ***HER2*** two helix binder described in the Examples below (SEQ ID NO.:8) has demonstrated an affinity of about 150 nM for its target,

```
      ***HER2***  .
ST   pair antiparallel alpha helix   ***Staphylococcal***     ***protein***
       ***A***   domain B
IT   Antibodies and Immunoglobulins
     RL: BSU (Biological study, unclassified); BIOL (Biological study)
        (IgG, anti-parallel alpha helixes capable of binding; two helix segment
        derived from   ***Staphylococcal***     ***protein***     ***A***
        domain B comprising a pair of anti-parallel alpha helixes capable of
        binding a target)
IT   neu (receptor)
     RL: BSU (Biological study, unclassified); BIOL (Biological study)
        (anti-parallel alpha helixes capable of binding; two helix segment
        derived from   ***Staphylococcal***     ***protein***     ***A***
        domain B comprising a pair of anti-parallel alpha helixes capable of
        binding a target)
IT   Diagnosis
        (mol.; two helix segment derived from   ***Staphylococcal***
          ***protein***     ***A***   domain B comprising a pair of anti-
parallel
        alpha helixes capable of binding a target)
IT   Disulfide group
        (two helix segment derived from Protein Z stabilized with; two helix
        segment derived from   ***Staphylococcal***     ***protein***
          ***A***   domain B comprising a pair of anti-parallel alpha helixes
        capable of binding a target)
IT   Biomarkers
     Protein sequences
     .alpha.-Helix
        (two helix segment derived from   ***Staphylococcal***
          ***protein***     ***A***   domain B comprising a pair of anti-
parallel
        alpha helixes capable of binding a target)
IT     ***Staphylococcal***     ***protein***     ***A***
     RL: BUU (Biological use, unclassified); DGN (Diagnostic use); PRP
     (Properties); BIOL (Biological study); USES (Uses)
        (two helix segment derived from   ***Staphylococcal***
          ***protein***     ***A***   domain B comprising a pair of anti-
parallel
        alpha helixes capable of binding a target)
IT   1031901-13-9
     RL: BUU (Biological use, unclassified); DGN (Diagnostic use); PRP
     (Properties); BIOL (Biological study); USES (Uses)
        (amino acid sequence, 35-residue; two helix segment derived from
          ***Staphylococcal***     ***protein***     ***A***   domain B
        comprising a pair of anti-parallel alpha helixes capable of binding a
        target)
IT   1031901-14-0
     RL: BUU (Biological use, unclassified); DGN (Diagnostic use); PRP
     (Properties); BIOL (Biological study); USES (Uses)
        (amino acid sequence, SEQ ID NO: 2 and SEQ ID NO. 3 combined; two helix
        segment derived from   ***Staphylococcal***     ***protein***
          ***A***   domain B comprising a pair of anti-parallel alpha helixes
        capable of binding a target)
IT   1031467-43-2   1031467-44-3
     RL: BUU (Biological use, unclassified); DGN (Diagnostic use); PRP
     (Properties); BIOL (Biological study); USES (Uses)
        (amino acid sequence, anti-  ***Her2***   two helix binder with
```

alternative substitutions with cysteine; two helix segment derived from ***Staphylococcal*** ***protein*** ***A*** domain B comprising a pair of anti-parallel alpha helixes capable of binding a target)

IT 1031467-42-1
RL: BUU (Biological use, unclassified); DGN (Diagnostic use); PRP (Properties); BIOL (Biological study); USES (Uses)
(amino acid sequence, anti- ***Her2*** two helix binder with preferred substitutions with cysteine; two helix segment derived from ***Staphylococcal*** ***protein*** ***A*** domain B comprising a pair of anti-parallel alpha helixes capable of binding a target)

IT 1031467-41-0
RL: BUU (Biological use, unclassified); DGN (Diagnostic use); PRP (Properties); BIOL (Biological study); USES (Uses)
(amino acid sequence, anti-IgG two helix binder with preferred substitutions with cysteine; two helix segment derived from ***Staphylococcal*** ***protein*** ***A*** domain B comprising a pair of anti-parallel alpha helixes capable of binding a target)

IT 1031901-16-2
RL: BUU (Biological use, unclassified); DGN (Diagnostic use); PRP (Properties); BIOL (Biological study); USES (Uses)
(amino acid sequence, representative anti- ***Her2*** two helix binder; two helix segment derived from ***Staphylococcal*** ***protein*** ***A*** domain B comprising a pair of anti-parallel alpha helixes capable of binding a target)

IT 1031901-15-1
RL: BUU (Biological use, unclassified); DGN (Diagnostic use); PRP (Properties); BIOL (Biological study); USES (Uses)
(amino acid sequence, representative anti-IgG two helix binder; two helix segment derived from ***Staphylococcal*** ***protein*** ***A*** domain B comprising a pair of anti-parallel alpha helixes capable of binding a target)

IT 1031468-61-7
RL: PRP (Properties)
(unclaimed protein sequence; two helix segment derived from ***Staphylococcal*** ***protein*** ***A*** domain B comprising a pair of anti-parallel alpha helixes capable of binding a target)

L14 ANSWER 6 OF 31 CAPLUS COPYRIGHT 2009 ACS on STN
AN 2008:731847 CAPLUS <<LOGINID::20090428>>
DN 150:140811
TI Affibody Molecules for In vivo Characterization of ***HER2*** -Positive Tumors by Near-Infrared Imaging
AU Lee, Sang Bong; Hassan, Moinuddin; Fisher, Robert; Chertov, Oleg; Chernomordik, Victor; Kramer-Marek, Gabriela; Gandjbakhche, Amir; Capala, Jacek
CS Radiation Oncology Branch, Center for Cancer Research, National Cancer Institute, National Institute of Child Health and Human Development, NIH, Bethesda, MD, 20892, USA
SO Clinical Cancer Research (2008), 14(12), 3840-3849
CODEN: CCREF4; ISSN: 1078-0432
PB American Association for Cancer Research
DT Journal

LA English

AB ***HER2*** overexpression has been assocd. with a poor prognosis and resistance to therapy in breast cancer patients. We are developing mol. probes for in vivo quant. imaging of ***HER2*** receptors using near-IR (NIR) optical imaging. The goal is to provide probes that will minimally interfere with the studied system, i.e., whose binding does not interfere with the binding of the therapeutic agents and whose effect on the target cells is minimal. We used three different types of ***HER2*** -specific Affibody mols. [monomer ZHER2:342, dimer (ZHER2:477)2, and albumin-binding domain-fused-(ZHER2:342)2] as targeting agents and labeled them with Alexa Fluor dyes. Trastuzumab was also conjugated, using com. available kits, as a std. control. The resulting conjugates were characterized in vitro by toxicity assays, Biacore affinity measurements, flow cytometry, and confocal microscopy. Semiquant. in vivo NIR optical imaging studies were carried out using mice with s.c. xenografts of ***HER2*** -pos. tumors. The ***HER2*** -specific Affibody mols. were not toxic to ***HER2*** -overexpressing cells and their binding to ***HER2*** did interfere with neither binding nor effectives of trastuzumab. The binding affinities and specificities of the Affibody-Alexa Fluor fluorescent conjugates to ***HER2*** were unchanged or minimally affected by the modifications. Pharmacokinetics and biodistribution studies showed the albumin-binding domain-fused-(ZHER2:342)2-Alexa Fluor 750 conjugate to be an optimal probe for optical imaging of ***HER2*** in vivo. Our results suggest that Affibody-Alexa Fluor conjugates may be used as a specific NIR probe for the noninvasive semiquant. imaging of ***HER2*** expression in vivo.

RE.CNT 46 THERE ARE 46 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

TI Affibody Molecules for In vivo Characterization of ***HER2*** -Positive Tumors by Near-Infrared Imaging

AB ***HER2*** overexpression has been assocd. with a poor prognosis and resistance to therapy in breast cancer patients. We are developing mol. probes for in vivo quant. imaging of ***HER2*** receptors using near-IR (NIR) optical imaging. The goal is to provide probes that will minimally interfere with the studied system,. . . binding of the therapeutic agents and whose effect on the target cells is minimal. We used three different types of ***HER2*** -specific Affibody mols. [monomer ZHER2:342, dimer (ZHER2:477)2, and albumin-binding domain-fused-(ZHER2:342)2] as targeting agents and labeled them with Alexa Fluor dyes. Trastuzumab. . . cytometry, and confocal microscopy. Semiquant. in vivo NIR optical imaging studies were carried out using mice with s.c. xenografts of ***HER2*** -pos. tumors. The ***HER2*** -specific Affibody mols. were not toxic to ***HER2*** -overexpressing cells and their binding to ***HER2*** did interfere with neither binding nor effectives of trastuzumab. The binding affinities and specificities of the Affibody-Alexa Fluor fluorescent conjugates to ***HER2*** were unchanged or minimally affected by the modifications. Pharmacokinetics and biodistribution studies showed the albumin-binding domain-fused-(ZHER2:342)2-Alexa Fluor 750 conjugate to be an optimal probe for optical imaging of ***HER2*** in vivo. Our results suggest that Affibody-Alexa Fluor conjugates may be used as a specific NIR probe for the noninvasive semiquant. imaging of ***HER2*** expression in vivo.

ST ***HER2*** receptor breast ovarian adenocarcinoma

IT Antitumor agents
Cell proliferation
Human
(Affibody mols. for in vivo characterization of ***HER2*** -pos.

tumors using near-IR imaging)
IT neu (receptor)
RL: ADV (Adverse effect, including toxicity); BSU (Biological study, unclassified); BIOL (Biological study)
(Affibody mols. for in vivo characterization of ***HER2*** -pos. tumors using near-IR imaging)
IT ***Staphylococcal*** ***protein*** ***A***
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(Affibody mols. for in vivo characterization of ***HER2*** -pos. tumors using near-IR imaging)
IT Imaging
(IR; Affibody mols. for in vivo characterization of ***HER2*** -pos. tumors using near-IR imaging)
IT Mammary gland, neoplasm
Ovary, neoplasm
(adenocarcinoma; Affibody mols. for in vivo characterization of ***HER2*** -pos. tumors using near-IR imaging)
IT Neuroglia, neoplasm
(glioblastoma; Affibody mols. for in vivo characterization of ***HER2*** -pos. tumors using near-IR imaging)
IT Adenocarcinoma
(mammary adenocarcinoma; Affibody mols. for in vivo characterization of ***HER2*** -pos. tumors using near-IR imaging)
IT Adenocarcinoma
(ovarian; Affibody mols. for in vivo characterization of ***HER2*** -pos. tumors using near-IR imaging)
IT 180288-69-1, Trastuzumab
RL: PAC (Pharmacological activity); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(Affibody mols. for in vivo characterization of ***HER2*** -pos. tumors using near-IR imaging)

L14 ANSWER 7 OF 31 CAPLUS COPYRIGHT 2009 ACS on STN
AN 2008:1435725 CAPLUS <<LOGINID::20090428>>
DN 150:50090
TI Effects of Lysine-Containing Mercaptoacetyl-Based Chelators on the Biodistribution of 99mTc-Labeled Anti- ***HER2*** Affibody Molecules
AU Tran, Thuy A.; Ekblad, Torun; Orlova, Anna; Sandstrom, Mattias; Feldwisch, Joachim; Wennborg, Anders; Abrahmsen, Lars; Tolmachev, Vladimir; Eriksson Karlstrom, Amelie
CS Unit of Biomedical Radiation Sciences, Rudbeck Laboratory Medical Radiation Physics, Uppsala University Hospital and Department of Medical Sciences, Uppsala University, Uppsala, Swed.
SO Bioconjugate Chemistry (2008), 19(12), 2568-2576
CODEN: BCCHES; ISSN: 1043-1802
PB American Chemical Society
DT Journal
LA English
AB The effects of polar (mercaptoacetyl-triseryl) and neg. charged (mercaptoacetyl-triglumatyl) chelators on the biodistribution of 99mTc-labeled anti- ***HER2*** Affibody mols. were previously investigated. With glycine, serine, and glutamate, we demonstrated that substitution with a single amino acid in the chelator can significantly influence the biodistribution properties and the excretion pathways. Here, we have taken this investigation further, by analyzing the effects of introduction of a pos. amino acid residue on the in vivo properties of the 99mTc-labeled Affibody mol. The Affibody mols. with

mercaptoacetyl-seryl-lysyl-seryl (maSKS) and mercaptoacetyl-trilysyl (maKKK) extensions were produced by peptide synthesis and labeled with 99mTc in alk. conditions. A comparative biodistribution was performed in normal mice to evaluate the excretion pathway. A shift toward renal excretion was obtained when serine was substituted with lysine in the chelating sequence. The radioactivity in the gastrointestinal tract was reduced 3-fold for the 99mTc-maSKS-ZHER2:342 and 99mTc-maKKK-ZHER2:342 in comparison with the 99mTc-maSSS- ZHER2:342 conjugate 4 h post injection (p.i.). The radioactivity in the liver was elevated when a triple substitution of pos. charged lysine was used. The tumor targeting properties of 99mTc-maSKS-ZHER2:342 were further investigated in SKOV-3 xenografts. The tumor uptake of 99mTc-maSKS-ZHER2:342 was 17 .+-. 7% IA/g 4 h p.i. Tumor xenografts were well-visualized by gamma scintigraphy. In conclusion, the substitution with one single lysine in the chelator results in better tumor imaging properties of the Affibody mol. ZHER2:342 and is favorable for imaging of tumors and metastases in the abdominal area. Multiple lysine residues in the chelator are, however, undesirable due to elevated uptake both in the liver and kidneys.

RE.CNT 33 THERE ARE 33 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

TI Effects of Lysine-Containing Mercaptoacetyl-Based Chelators on the Biodistribution of 99mTc-Labeled Anti- ***HER2*** Affibody Molecules

AB The effects of polar (mercaptoacetyl-triseryl) and neg. charged (mercaptoacetyl-triglumatyl) chelators on the biodistribution of 99mTc-labeled anti- ***HER2*** Affibody mols. were previously investigated. With glycine, serine, and glutamate, we demonstrated that substitution with a single amino acid in. . .

ST lysine mercaptoacetyl chelator technetium 99m ***HER2*** affibody pharmacokinetics

IT ***Staphylococcal*** ***protein*** ***A***
RL: DGN (Diagnostic use); PKT (Pharmacokinetics); BIOL (Biological study); USES (Uses)
(Z-domain synthetic homologues; lysine-contg. mercaptoacetyl chelators effect on 99mTc-labeled anti- ***HER2*** affibody pharmacokinetics)

IT Chelating agents
Human
Pharmacokinetics
Scintigraphic agents
Scintigraphy
Structure-activity relationship
(lysine-contg. mercaptoacetyl chelators effect on 99mTc-labeled anti- ***HER2*** affibody pharmacokinetics)

IT neu (receptor)
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(lysine-contg. mercaptoacetyl chelators effect on 99mTc-labeled anti- ***HER2*** affibody pharmacokinetics)

IT Imaging
(tumor; lysine-contg. mercaptoacetyl chelators effect on 99mTc-labeled anti- ***HER2*** affibody pharmacokinetics)

IT 378784-45-3DP, Technetium 99m, chelator-anti- ***HER2*** affibody conjugate labeled with, biological studies 1056015-90-7DP, anti- ***HER2*** affibody conjugate, 99mTc labeled 1093184-00-9DP, anti- ***HER2*** affibody conjugate, 99mTc labeled 1093184-01-0DP, anti- ***HER2*** affibody conjugate, 99mTc labeled
RL: DGN (Diagnostic use); PKT (Pharmacokinetics); SPN (Synthetic preparation); BIOL (Biological study); PREP (Preparation); USES (Uses)
(lysine-contg. mercaptoacetyl chelators effect on 99mTc-labeled anti-

HER2 affibody pharmacokinetics)

L14 ANSWER 8 OF 31 CAPLUS COPYRIGHT 2009 ACS on STN
AN 2008:1265386 CAPLUS <<LOGINID::20090428>>
DN 149:582162
TI Specific Targeting of ***HER2*** Overexpressing Breast Cancer Cells with Doxorubicin-Loaded Trastuzumab-Modified Human Serum Albumin Nanoparticles
AU Anhorn, Marion G.; Wagner, Sylvia; Kreuter, Joerg; Langer, Klaus; von Briesen, Hagen
CS Department of Cell Biology and Applied Virology, Fraunhofer Institute for Biomedical Engineering, St. Ingbert, 66386, Germany
SO Bioconjugate Chemistry (2008), 19(12), 2321-2331
CODEN: BCCHES; ISSN: 1043-1802
PB American Chemical Society
DT Journal
LA English
AB Specific targeting of tumor cells to achieve higher drug levels in tumor tissue and to overcome cardiotoxic and other secondary effects is the major goal in cancer therapy. With trastuzumab as a humanized monoclonal antibody binding, the ***HER2*** receptor specific targeting is possible. In the present study, target-oriented nanoparticles based on biodegradable human serum albumin (HSA) loaded with cytostatic drug doxorubicin were developed. The surface of the nanoparticles was modified by covalent attachment of trastuzumab. ***HER2*** overexpressing breast cancer cells showed a good cellular binding and uptake of these nanoparticles. The specific transport of the cytostatic drug doxorubicin with this nanoparticulate formulation into the ***HER2*** overexpressing breast cancer cells, their release, and biol. activity was demonstrated. The results indicate that these cell-type specific drug-loaded nanoparticles could achieve an improvement in cancer therapy. To our knowledge, this is the first study demonstrating a specific trastuzumab-based targeting of ***HER2*** overexpressing breast cancer cells with doxorubicin-loaded nanoparticles.
RE.CNT 31 THERE ARE 31 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT
TI Specific Targeting of ***HER2*** Overexpressing Breast Cancer Cells with Doxorubicin-Loaded Trastuzumab-Modified Human Serum Albumin Nanoparticles
AB . . . and other secondary effects is the major goal in cancer therapy. With trastuzumab as a humanized monoclonal antibody binding, the ***HER2*** receptor specific targeting is possible. In the present study, target-oriented nanoparticles based on biodegradable human serum albumin (HSA) loaded with cytostatic drug doxorubicin were developed. The surface of the nanoparticles was modified by covalent attachment of trastuzumab. ***HER2*** overexpressing breast cancer cells showed a good cellular binding and uptake of these nanoparticles. The specific transport of the cytostatic drug doxorubicin with this nanoparticulate formulation into the ***HER2*** overexpressing breast cancer cells, their release, and biol. activity was demonstrated. The results indicate that these cell-type specific drug-loaded nanoparticles. . . achieve an improvement in cancer therapy. To our knowledge, this is the first study demonstrating a specific trastuzumab-based targeting of ***HER2*** overexpressing breast cancer cells with doxorubicin-loaded nanoparticles.
ST targeting breast cancer doxorubicin trastuzumab albumin nanoparticle ***HER2*** targeting
IT Antibodies and Immunoglobulins

RL: BSU (Biological study, unclassified); PRP (Properties); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(IgG; specific targeting of ***HER2*** overexpressing breast cancer cells with doxorubicin-loaded trastuzumab-modified human serum albumin nanoparticles)

IT Albumins, biological studies
RL: BSU (Biological study, unclassified); PRP (Properties); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(serum; specific targeting of ***HER2*** overexpressing breast cancer cells with doxorubicin-loaded trastuzumab-modified human serum albumin nanoparticles)

IT Antitumor agents
Human
Mammary gland, neoplasm
Particle size
Polydispersity
Surface treatment
Zeta potential
(specific targeting of ***HER2*** overexpressing breast cancer cells with doxorubicin-loaded trastuzumab-modified human serum albumin nanoparticles)

IT Drug delivery systems
(targeted; specific targeting of ***HER2*** overexpressing breast cancer cells with doxorubicin-loaded trastuzumab-modified human serum albumin nanoparticles)

IT Biological transport
(uptake; specific targeting of ***HER2*** overexpressing breast cancer cells with doxorubicin-loaded trastuzumab-modified human serum albumin nanoparticles)

IT 23214-92-8, Doxorubicin
RL: BSU (Biological study, unclassified); PAC (Pharmacological activity); PRP (Properties); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(specific targeting of ***HER2*** overexpressing breast cancer cells with doxorubicin-loaded trastuzumab-modified human serum albumin nanoparticles)

IT 174569-25-6D, Mpeg- ***spa*** , complex with human serum albumin nanoparticles 180288-69-1, Herceptin 357277-60-2D, complex with Herceptin and human serum albumin nanoparticles 357277-60-2D, complex with human serum albumin nanoparticles
RL: BSU (Biological study, unclassified); PRP (Properties); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(specific targeting of ***HER2*** overexpressing breast cancer cells with doxorubicin-loaded trastuzumab-modified human serum albumin nanoparticles)

L14 ANSWER 9 OF 31 CAPLUS COPYRIGHT 2009 ACS on STN
AN 2008:488084 CAPLUS <<LOGINID::20090428>>
TI [18F]FBEM-ZHER2:342-Affibody molecule-a new molecular tracer for in vivo monitoring of ***HER2*** expression by positron emission tomography
AU Kramer-Marek, Gabriela; Kiesewetter, Dale O.; Martiniova, Lucia; Jagoda, Elaine; Lee, Sang Bong; Capala, Jacek
CS National Cancer Institute, National Institutes of Health, Bethesda, MD, 20892, USA
SO European Journal of Nuclear Medicine and Molecular Imaging (2008), 35(5), 1008-1018
CODEN: EJNMA6; ISSN: 1619-7070

PB Springer
DT Journal
LA English
AB The expression of ***human*** ***epidermal*** ***growth*** ***factor*** ***receptor*** - ***2*** (***HER2***) receptors in cancers is correlated with a poor prognosis. If assessed in vivo, it could be used for selection of appropriate therapy for individual patients and for monitoring of the tumor response to targeted therapies. We have radiolabeled a ***HER2*** -binding Affibody mol. with fluorine-18 for in vivo monitoring of the ***HER2*** expression by positron emission tomog. (PET). The ***HER2*** -binding ZHER2:342-Cys Affibody mol. was conjugated with N-(2-(4-[18F]fluorobenzamido)ethyl)maleimide ([18F]FBEM). The in vitro binding of the resulting radioconjugate was characterized by receptor satn. and competition assays. For in vivo studies, the radioconjugate was injected into the tail vein of mice bearing s.c. ***HER2*** -pos. or ***HER2*** -neg. tumors. Some of the mice were pre-treated with non-labeled ZHER2:342-Cys. The animals were sacrificed at different times post-injection, and the radioactivity in selected tissues was measured. PET images were obtained using an animal PET scanner. In vitro expts. indicated specific, high-affinity binding to ***HER2*** . PET imaging revealed a high accumulation of the radioactivity in the tumor as early as 20 min after injection, with a plateau being reached after 60 min. These results were confirmed by biodistribution studies demonstrating that, as early as 1 h post-injection, the tumor to blood concn. ratio was 7.5 and increased to 27 at 4 h. Pre-satn. of the receptors with unlabeled ZHER2:342-Cys lowered the accumulation of radioactivity in ***HER2*** -pos. tumors to the levels obsd. in ***HER2*** -neg. ones. Our results suggest that the [18F]FBEM-ZHER2:342 radioconjugate can be used to assess ***HER2*** expression in vivo.
RE.CNT 38 THERE ARE 38 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT
TI [18F]FBEM-ZHER2:342-Affibody molecule-a new molecular tracer for in vivo monitoring of ***HER2*** expression by positron emission tomography
AB The expression of ***human*** ***epidermal*** ***growth*** ***factor*** ***receptor*** - ***2*** (***HER2***) receptors in cancers is correlated with a poor prognosis. If assessed in vivo, it could be used for selection of appropriate therapy for individual patients and for monitoring of the tumor response to targeted therapies. We have radiolabeled a ***HER2*** -binding Affibody mol. with fluorine-18 for in vivo monitoring of the ***HER2*** expression by positron emission tomog. (PET). The ***HER2*** -binding ZHER2:342-Cys Affibody mol. was conjugated with N-(2-(4-[18F]fluorobenzamido)ethyl)maleimide ([18F]FBEM). The in vitro binding of the resulting radioconjugate was characterized by receptor. . . satn. and competition assays. For in vivo studies, the radioconjugate was injected into the tail vein of mice bearing s.c. ***HER2*** -pos. or ***HER2*** -neg. tumors. Some of the mice were pre-treated with non-labeled ZHER2:342-Cys. The animals were sacrificed at different times post-injection, and the. . . tissues was measured. PET images were obtained using an animal PET scanner. In vitro expts. indicated specific, high-affinity binding to ***HER2*** . PET imaging revealed a high accumulation of the radioactivity in the tumor as early as 20 min after injection, with. . . and increased to 27 at 4 h. Pre-satn. of the receptors with unlabeled ZHER2:342-Cys lowered the accumulation of radioactivity in ***HER2*** -pos. tumors to the levels obsd. in ***HER2*** -neg. ones. Our results suggest that the [18F]FBEM-ZHER2:342 radioconjugate can be used to assess ***HER2***

expression in vivo.
ST flourine 18 FBEM affibody ***HER2*** receptor PET biodistribution
IT INDEXING IN PROGRESS
IT INDEXING IN PROGRESS
IT ***Staphylococcal*** ***protein*** ***A***
RL: DGN (Diagnostic use); PKT (Pharmacokinetics); SPN (Synthetic preparation); BIOL (Biological study); PREP (Preparation); USES (Uses)
(ZHER2:342-[18F]FBEM conjugates; [18F]FBEM-ZHER2:342-Affibody mol. as new mol. tracer for in vivo monitoring of ***HER2*** expression by positron emission tomog.)
IT Human
Mammary gland, neoplasm
Ovary, neoplasm
Positron-emission tomography
([18F]FBEM-ZHER2:342-Affibody mol. as new mol. tracer for in vivo monitoring of ***HER2*** expression by positron emission tomog.)
IT neu (receptor)
RL: BSU (Biological study, unclassified); BIOL (Biological study)
([18F]FBEM-ZHER2:342-Affibody mol. as new mol. tracer for in vivo monitoring of ***HER2*** expression by positron emission tomog.)
IT Imaging agents
(tomog. contrast agents; [18F]FBEM-ZHER2:342-Affibody mol. as new mol. tracer for in vivo monitoring of ***HER2*** expression by positron emission tomog.)
IT Imaging
(tumor; [18F]FBEM-ZHER2:342-Affibody mol. as new mol. tracer for in vivo monitoring of ***HER2*** expression by positron emission tomog.)

L14 ANSWER 10 OF 31 CAPLUS COPYRIGHT 2009 ACS on STN
AN 2008:1081483 CAPLUS <<LOGINID::20090428>>
DN 150:16162
TI Radiolabeling of ***HER2*** -specific Affibody molecule with F-18
AU Kiesewetter, Dale O.; Kraemer-Marek, Gabriela; Ma, Ying; Capala, Jacek
CS Positron Emission Tomography Radiochemistry Group, NIBIB, Bethesda, MD, 20892, USA
SO Journal of Fluorine Chemistry (2008), 129(9), 799-806
CODEN: JFLCAR; ISSN: 0022-1139
PB Elsevier B.V.
DT Journal
LA English
AB The presence of human epidermal growth factor type 2 (***HER2***) on 20-30% of human breast cancer is a prognostic indicator of more rapid disease progression and a therapeutic indicator for anti- ***HER2*** monoclonal antibodies. Because the literature has demonstrated some discordance between primary and metastatic tumors in the same patient for expression of the ***HER2*** marker, we set out to develop an imaging agent that could be used to assess the marker concn. in vivo in an individual patient. The pharmaceutical company Affibody AB has optimized the specificity of Affibody mols. for ***HER2*** . Two Affibody mols., a 7 kDa and an 8 kDa protein, were designed with a single carboxy terminal cysteine in order to provide a specific location for the purposes of labeling for various types of imaging. We have prepd. [18F]FBEM utilizing a coupling reaction between [18F]fluorobenzoic acid and aminoethylmaleimide. We then optimized the conjugation of this radiolabeled maleimide to the free sulfhydryl of cysteine by incubating at pH 7.4 in phosphate buffered saline contg. 0.1% sodium ascorbate. An

overall uncorrected yield of radiolabeled Affibody mol. of approx. 10% from [18F]fluoride was achieved in a 2 h synthesis. These conjugated Affibody mols. were obtained with a specific activity of 2.51 .+-. 0.92 MBq/.mu.g. Characterization of the product by HPLC-MS supported the conjugation of [18F]FBEM with the Affibody mol. The radiolabeled Affibody mol. retained its binding specificity as demonstrated by successful imaging of xenografts expressing ***HER2*** .

RE.CNT 24 THERE ARE 24 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

TI Radiolabeling of ***HER2*** -specific Affibody molecule with F-18

AB The presence of human epidermal growth factor type 2 (***HER2***) on 20-30% of human breast cancer is a prognostic indicator of more rapid disease progression and a therapeutic indicator for anti- ***HER2*** monoclonal antibodies. Because the literature has demonstrated some discordance between primary and metastatic tumors in the same patient for expression of the ***HER2*** marker, we set out to develop an imaging agent that could be used to assess the marker concn. in vivo in an individual patient. The pharmaceutical company Affibody AB has optimized the specificity of Affibody mols. for ***HER2*** . Two Affibody mols., a 7 kDa and an 8 kDa protein, were designed with a single carboxy terminal cysteine in. . . with the Affibody mol. The radiolabeled Affibody mol. retained its binding specificity as demonstrated by successful imaging of xenografts expressing ***HER2*** .

ST fluorine radiodiagnosis agent ***HER2*** tumor marker cancer Affibody PET

IT Mass spectrometry
(HPLC combined with; radiolabeling of ***HER2*** -specific Affibody mol. with F-18)

IT ***Staphylococcal*** ***protein*** ***A***
RL: ARU (Analytical role, unclassified); BUU (Biological use, unclassified); ANST (Analytical study); BIOL (Biological study); USES (Uses)
(Z-domain synthetic homologues; ZHER2-342-cys and ZHER2-3395-cys; radiolabeling of ***HER2*** -specific Affibody mol. with F-18)

IT Diagnosis
(cancer; radiolabeling of ***HER2*** -specific Affibody mol. with F-18)

IT Proteins
RL: ADV (Adverse effect, including toxicity); ANT (Analyte); BSU (Biological study, unclassified); DGN (Diagnostic use); ANST (Analytical study); BIOL (Biological study); USES (Uses)
(conjugates, 18F-Affibody; radiolabeling of ***HER2*** -specific Affibody mol. with F-18)

IT HPLC
(mass spectrometry combined with; radiolabeling of ***HER2*** -specific Affibody mol. with F-18)

IT Diagnosis
(radiodiagnosis; radiolabeling of ***HER2*** -specific Affibody mol. with F-18)

IT Diagnostic agents
Electron ionization mass spectrometry
Human
Mammary gland, neoplasm
Positron-emission tomography
Prognosis
Reversed phase HPLC
Tumor markers

(radiolabeling of ***HER2*** -specific Affibody mol. with F-18)
IT neu (receptor)
RL: ADV (Adverse effect, including toxicity); ANT (Analyte); BSU (Biological study, unclassified); DGN (Diagnostic use); ANST (Analytical study); BIOL (Biological study); USES (Uses)
(radiolabeling of ***HER2*** -specific Affibody mol. with F-18)
IT 13981-56-1, biological studies
RL: ARG (Analytical reagent use); BUU (Biological use, unclassified); ANST (Analytical study); BIOL (Biological study); USES (Uses)
(radiolabeling of ***HER2*** -specific Affibody mol. with F-18)
IT 127885-65-8, NAP 5
RL: ARG (Analytical reagent use); BUU (Biological use, unclassified); DGN (Diagnostic use); ANST (Analytical study); BIOL (Biological study); USES (Uses)
(radiolabeling of ***HER2*** -specific Affibody mol. with F-18)
IT 929706-89-8P
RL: ARG (Analytical reagent use); BUU (Biological use, unclassified); DGN (Diagnostic use); SPN (Synthetic preparation); ANST (Analytical study); BIOL (Biological study); PREP (Preparation); USES (Uses)
(radiolabeling of ***HER2*** -specific Affibody mol. with F-18)
IT 2942-58-7, Diethylcyanophosphonate 10011-97-9 51805-45-9 125923-10-6 1089194-09-1
RL: RCT (Reactant); RACT (Reactant or reagent)
(radiolabeling of ***HER2*** -specific Affibody mol. with F-18)

L14 ANSWER 11 OF 31 CAPLUS COPYRIGHT 2009 ACS on STN
AN 2008:61445 CAPLUS <<LOGINID::20090428>>
DN 148:277515
TI Modification of adenovirus capsid with a designed protein ligand yields a gene vector targeted to a major molecular marker of cancer
AU Belousova, Natalya; Mikheeva, Galina; Gelovani, Juri; Krasnykh, Victor
CS Department of Experimental Diagnostic Imaging, The University of Texas M. D. Anderson Cancer Center, Houston, TX, 77030, USA
SO Journal of Virology (2008), 82(2), 630-637
CODEN: JOVIAM; ISSN: 0022-538X
PB American Society for Microbiology
DT Journal
LA English
AB The future of genetic interventions in humans critically depends on the selectivity and efficiency of gene transfer to target tissues. The viral gene vectors explored to date cannot selectively transduce the desired targets. While substantial progress has been made in developing targeting strategies for adenovirus (Ad) vectors, future advances in this direction are severely limited by the shortage of naturally existing mols. available for use as targeting ligands. This shortage is due to fundamental and irresolvable differences at the level of both posttranslational modifications and intracellular trafficking between the Ad structural proteins and those natural proteins that are involved in interactions with the cell surface and could otherwise be considered as potential targeting ligands. We hypothesized that this problem could be resolved by altering the natural tropism of Ad vector through incorporation into its capsid of a rationally designed protein ligand, an affibody, whose structural, functional, and biosynthetic properties make it compatible with the Ad assembly process. We tested this hypothesis by redesigning the receptor-binding Ad protein, the fiber, using affibodies specific for human epidermal growth factor receptor type 2 (***Her2***), a major mol. marker of human tumors. The biosynthesis and folding of these fiber

chimeras were fully compatible with Ad virion formation, and the resultant viral vectors were capable of selective delivery of a dual-function transgene to ***Her2*** -expressing cancer cells. By establishing the feasibility of this affibody-based approach to Ad vector targeting, the present study lays the foundation for further development of Ad vector technol. toward its clin. use.

RE.CNT 34 THERE ARE 34 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

AB . . . hypothesis by redesigning the receptor-binding Ad protein, the fiber, using affibodies specific for human epidermal growth factor receptor type 2 (***Her2***), a major mol. marker of human tumors. The biosynthesis and folding of these fiber chimeras were fully compatible with Ad virion formation, and the resultant viral vectors were capable of selective delivery of a dual-function transgene to ***Her2*** -expressing cancer cells. By establishing the feasibility of this affibody-based approach to Ad vector targeting, the present study lays the foundation. . .

ST . . . chimeric protein fiber fibritin affibody adenoviral vector; adenovirus 5 genetic vector chimeric protein fiber; gene therapy recombinant adenoviral vector tropism ***Her2*** ; genetic engineering Ad5 genetic vector tropism neu

IT Gene, microbial
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(11F. ***Her2*** :4, synthetic chimeric construct; modification of adenovirus capsid with designed protein ligand yields genetic vector targeted to major mol. marker of cancer)

IT Gene, microbial
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(11F. ***Her2*** :7, synthetic chimeric construct; modification of adenovirus capsid with designed protein ligand yields genetic vector targeted to major mol. marker of cancer)

IT Human
(293A cells expression ***Her2*** ; modification of adenovirus capsid with designed protein ligand yields genetic vector targeted to major mol. marker of cancer)

IT Gene, microbial
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(FF. ***Her2*** :4, synthetic chimeric construct; modification of adenovirus capsid with designed protein ligand yields genetic vector targeted to major mol. marker of cancer)

IT Gene, microbial
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(FF. ***Her2*** :7, synthetic chimeric construct; modification of adenovirus capsid with designed protein ligand yields genetic vector targeted to major mol. marker of cancer)

IT neu (receptor)
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(***Her2*** , adenoviral vector targeted to; modification of adenovirus capsid with designed protein ligand yields genetic vector targeted to major mol. marker of cancer)

IT ***Staphylococcal*** ***protein*** ***A***
RL: BSU (Biological study, unclassified); PRP (Properties); THU (Therapeutic use); BIOL (Biological study); USES (Uses)

(adenoviral fiber protein fusion protein with; modification of adenovirus capsid with designed protein ligand yields genetic vector targeted to major mol. marker of cancer)

IT Animal cell line
(***her2*** -expressing 293A cells, delivery of transgene to; modification of adenovirus capsid with designed protein ligand yields genetic vector targeted to major mol. marker of cancer)

IT Genetic engineering
(of adenoviral vector with tropism for ***Her2*** -expressing cancer cells; modification of adenovirus capsid with designed protein ligand yields genetic vector targeted to major mol. marker of cancer)

L14 ANSWER 12 OF 31 CAPLUS COPYRIGHT 2009 ACS on STN
AN 2008:1387552 CAPLUS <<LOGINID::20090428>>
DN 150:136254
TI Dimeric ***HER2*** -specific affibody molecules inhibit proliferation of the SKBR-3 breast cancer cell line
AU Ekerljung, Lina; Lindborg, Malin; Gedda, Lars; Frejd, Fredrik Y.; Carlsson, Joergen; Lennartsson, Johan
CS Department of Oncology, Radiology and Clinical Immunology, Division of Biomedical Radiation Sciences, Rudbeck Laboratory, Uppsala University, Uppsala, SE-751 85, Swed.
SO Biochemical and Biophysical Research Communications (2008), 377(2), 489-494
CODEN: BBRCA9; ISSN: 0006-291X
PB Elsevier Inc.
DT Journal
LA English
AB ***HER2*** -specific affibody mols. in different formats have previously been shown to be useful tumor targeting agents for radionuclide-based imaging and therapy applications, but their biol. effect on tumor cells is not well known. In this study, two dimeric ((ZHER2:4)2 and (ZHER2:342)2) and one monomeric (ZHER2:342) ***HER2*** -specific affibody mols. are investigated with respect to biol. activity. Both (ZHER2:4)2 and (ZHER2:342)2 were found to decrease the growth rate of SKBR-3 cells to the same extent as the antibody trastuzumab. When the substances were removed, the cells treated with the dimeric affibody mols. continued to be growth suppressed while the cells treated with trastuzumab immediately resumed normal proliferation. The effects of ZHER2:342 were minor on both proliferation and cell signaling. The dimeric (ZHER2:4)2 and (ZHER2:342)2 both reduced growth of SKBR-3 cells and may prove therapeutically useful either by themselves or as carriers of radionuclides or other cytotoxic agents.
RE.CNT 22 THERE ARE 22 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT
TI Dimeric ***HER2*** -specific affibody molecules inhibit proliferation of the SKBR-3 breast cancer cell line
AB ***HER2*** -specific affibody mols. in different formats have previously been shown to be useful tumor targeting agents for radionuclide-based imaging and therapy. . . effect on tumor cells is not well known. In this study, two dimeric ((ZHER2:4)2 and (ZHER2:342)2) and one monomeric (ZHER2:342) ***HER2*** -specific affibody mols. are investigated with respect to biol. activity. Both (ZHER2:4)2 and (ZHER2:342)2 were found to decrease the growth rate. . .
ST antitumor ***HER2*** specific affibody breast cancer
IT ***Staphylococcal*** ***protein*** ***A***
RL: DMA (Drug mechanism of action); PAC (Pharmacological activity); THU

(Therapeutic use); BIOL (Biological study); USES (Uses)
(***HER2*** -specific affibody; dimeric ***HER2*** -specific affibody mols. inhibit proliferation of the SKBR-3 breast cancer cell line)

IT Phosphorylation, biological
(autophosphorylation, of ***HER2*** ; dimeric ***HER2*** -specific affibody mols. inhibit proliferation of the SKBR-3 breast cancer cell line)

IT Antitumor agents
Drug targets
Human
Mammary gland, neoplasm
Signal transduction
(dimeric ***HER2*** -specific affibody mols. inhibit proliferation of the SKBR-3 breast cancer cell line)

IT Cell proliferation
(inhibition; dimeric ***HER2*** -specific affibody mols. inhibit proliferation of the SKBR-3 breast cancer cell line)

IT neu (receptor)
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(target; dimeric ***HER2*** -specific affibody mols. inhibit proliferation of the SKBR-3 breast cancer cell line)

IT 180288-69-1, Trastuzumab 867229-20-7D, Protein Zher2:4 (synthetic), dimers 1046467-67-7, Protein ZHER2:342 (synthetic) 1046467-67-7D, Protein ZHER2:342 (synthetic), dimers
RL: DMA (Drug mechanism of action); PAC (Pharmacological activity); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(dimeric ***HER2*** -specific affibody mols. inhibit proliferation of the SKBR-3 breast cancer cell line)

IT 142243-02-5 148640-14-6, Akt protein kinase
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(phosphorylation; dimeric ***HER2*** -specific affibody mols. inhibit proliferation of the SKBR-3 breast cancer cell line)

L14 ANSWER 13 OF 31 CAPLUS COPYRIGHT 2009 ACS on STN DUPLICATE 2
AN 2008:61251 CAPLUS <<LOGINID::20090428>>
DN 148:300585
TI Simplified characterization through site-specific protease-mediated release of affinity proteins selected by staphylococcal display
AU Kronqvist, Nina; Loefblom, John; Severa, Denise; Staahl, Stefan; Wernerus, Henrik
CS Department of Molecular Biotechnology, School of Biotechnology, Royal Institute of Technology (KTH), AlbaNova University Center, Stockholm, Swed.
SO FEMS Microbiology Letters (2008), 278(1), 128-136
CODEN: FMLED7; ISSN: 0378-1097
PB Blackwell Publishing Ltd.
DT Journal
LA English
AB The prodn. of candidate affinity proteins in a sol. form, for downstream characterization, is often a time-consuming step in combinatorial protein engineering methods. Here, a novel approach for efficient prodn. of candidate clones is described based on direct cleavage of the affinity protein from the surface of Staphylococcus carnosus, followed by affinity purifn. To find a suitable strategy, three new fusion protein constructs were created, introducing a protease site for specific cleavage and purifn. tags for affinity chromatog. purifications into the staphylococcal

display vector. The three modified strains were evaluated in terms of transformation frequency, surface expression level and protease cleavage efficiency. A protocol for efficient affinity purifn. of protease-released affinity proteins using the introduced fusion-tags was successfully used, and the functionality of protease-treated and purified proteins was verified in a biosensor assay. To evaluate the devised method, a previously selected ***HER2*** -specific affibody was produced applying the new principle and was used to analyze ***HER2*** expression on human breast cancer cells.

RE.CNT 30 THERE ARE 30 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

AB . . . functionality of protease-treated and purified proteins was verified in a biosensor assay. To evaluate the devised method, a previously selected ***HER2*** -specific affibody was produced applying the new principle and was used to analyze ***HER2*** expression on human breast cancer cells.

IT ***Staphylococcal*** ***protein*** ***A***
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(fusion protein with ***human*** ***epidermal*** ***growth*** ***factor*** ***receptor*** ***2*** ; simplified characterization through site-specific protease-mediated release of affinity proteins selected by staphylococcal display)

L14 ANSWER 14 OF 31 CAPLUS COPYRIGHT 2009 ACS on STN
AN 2008:1116240 CAPLUS <<LOGINID::20090428>>
TI Affinity-based entrapment of the ***HER2*** receptor in the endoplasmic reticulum using an affibody molecule
AU Vernet, Erik; Konrad, Anna; Lundberg, Emma; Nygren, Per-Aake; Graeslund, Torbjoern
CS Department of Molecular Biotechnology, Albanova University Center, Royal Institute of Technology (KTH), Stockholm, SE-106 91, Swed.
SO Journal of Immunological Methods (2008), 338(1-2), 1-6
CODEN: JIMMBG; ISSN: 0022-1759
PB Elsevier B.V.
DT Journal
LA English
AB Interference with the export of cell surface receptors can be performed through co-expression of specific affinity mols. designed for entrapment in the endoplasmic reticulum during the export process. We describe the investigation of a small (6 kDa) non-Ig-based ***HER2*** receptor binding affibody mol. (ZHER2:00477), for use in affinity mediated entrapment of the ***HER2*** receptor in the ER. Constructs encoding ZHER2:00477 or a control affibody protein, with or without ER-retention peptide extensions (KDEL), were expressed in the ***HER2*** over-expressing cell line SKOV-3. Intracellular expression of the full-length affibody constructs could be confirmed by probing cell exts. by Western blotting. Confocal immunofluorescence microscopy expts. showed extensive co-localization of the ***HER2*** receptor and ZHER2:00477-KDEL in the ER, whereas the use of a KDEL-extended control affibody mol. resulted in distinct and sep. signals from cell surface-localized ***HER2*** receptor and ER-localized affibody protein. This indicated a capability of the ZHER2:00477-KDEL fusion protein to functionally interfere with the export process of ***HER2*** receptor in a specific manner. Using flow cytometry and cell proliferation analyses, it could be shown that expression of the ZHER2:00477-KDEL fusion construct in the SKOV-3 cell line resulted both in a marked redn. in cell surface level of ***HER2*** receptors and that

the cell population doubling time was significantly increased. Expression of the ZHER2:00477-KDEL fusion protein in addnl. cell lines of different origin and with different expression levels of endogenous ***HER2*** receptor compared to SKOV-3, also resulted in depletion of the cell surface levels of ***HER2*** receptor. This indicated upon a general ability of the ZHER2:00477-KDEL fusion protein to functionally interfere with the export process of ***HER2*** .

RE.CNT 35 THERE ARE 35 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

TI Affinity-based entrapment of the ***HER2*** receptor in the endoplasmic reticulum using an affibody molecule

AB . . . for entrapment in the endoplasmic reticulum during the export process. We describe the investigation of a small (6 kDa) non-Ig-based ***HER2*** receptor binding affibody mol. (ZHER2:00477), for use in affinity mediated entrapment of the ***HER2*** receptor in the ER. Constructs encoding ZHER2:00477 or a control affibody protein, with or without ER-retention peptide extensions (KDEL), were expressed in the ***HER2*** over-expressing cell line SKOV-3. Intracellular expression of the full-length affibody constructs could be confirmed by probing cell exts. by Western blotting. Confocal immunofluorescence microscopy expts. showed extensive co-localization of the ***HER2*** receptor and ZHER2:00477-KDEL in the ER, whereas the use of a KDEL-extended control affibody mol. resulted in distinct and sep. signals from cell surface-localized ***HER2*** receptor and ER-localized affibody protein. This indicated a capability of the ZHER2:00477-KDEL fusion protein to functionally interfere with the export process of ***HER2*** receptor in a specific manner. Using flow cytometry and cell proliferation analyses, it could be shown that expression of the ZHER2:00477-KDEL fusion construct in the SKOV-3 cell line resulted both in a marked redn. in cell surface level of ***HER2*** receptors and that the cell population doubling time was significantly increased. Expression of the ZHER2:00477-KDEL fusion protein in addnl. cell lines of different origin and with different expression levels of endogenous ***HER2*** receptor compared to SKOV-3, also resulted in depletion of the cell surface levels of ***HER2*** receptor. This indicated upon a general ability of the ZHER2:00477-KDEL fusion protein to functionally interfere with the export process of ***HER2*** .

ST entrapment ***HER2*** receptor endoplasmic reticulum affibody

IT INDEXING IN PROGRESS

IT INDEXING IN PROGRESS

IT Proteins
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(affibodies, fusion protein with KDEL; affinity-based entrapment of ***HER2*** receptor in endoplasmic reticulum using affibody fusion protein with KDEL)

IT Fusion proteins (chimeric proteins)
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(affibody with KDEL; affinity-based entrapment of ***HER2*** receptor in endoplasmic reticulum using affibody fusion protein with KDEL)

IT Cell proliferation
Endoplasmic reticulum
Human
(affinity-based entrapment of ***HER2*** receptor in endoplasmic reticulum using affibody fusion protein with KDEL)

IT neu (receptor)
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(affinity-based entrapment of ***HER2*** receptor in endoplasmic reticulum using affibody fusion protein with KDEL)
IT Biological transport
(export; affinity-based entrapment of ***HER2*** receptor in endoplasmic reticulum using affibody fusion protein with KDEL)
IT ***Staphylococcal*** ***protein*** ***A***
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(fusion with KDEL; affinity-based entrapment of ***HER2*** receptor in endoplasmic reticulum using affibody fusion protein with KDEL)

L14 ANSWER 15 OF 31 CAPLUS COPYRIGHT 2009 ACS on STN
AN 2007:14198 CAPLUS <<LOGINID::20090428>>
DN 146:99124
TI Antibody-immunostimulant fusion constructs as effective adjuvants for protein vaccination
IN Penichet, Manuel L.; Helguera, Gustavo F.; Morrison, Sherie L.
PA The Regents of the University of California, USA
SO U.S. Pat. Appl. Publ., 56pp., Cont.-in-part of U.S. Ser. No. 118,473.
CODEN: USXXCO
DT Patent
LA English
FAN.CNT 2

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	US 20070003514	A1	20070104	US 2005-193982	20050729
	US 20030187225	A1	20031002	US 2002-118473	20020405
	WO 2003080106	A1	20031002	WO 2003-US9136	20030321
	W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW				
	RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
	WO 2007016185	A2	20070208	WO 2006-US29077	20060726
	WO 2007016185	A3	20070920		
	W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW				
	RW: AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG, BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AP, EA, EP, OA				
PRAI	US 2002-118473	A2	20020405		
	WO 2003-US9136	A2	20030321		
	US 2005-692059P	P	20050616		

US 2002-366917P P 20020321
US 2005-193982 A 20050729

AB The authors disclose the use of antibody-immunostimulant fusion proteins as adjuvants for antigenic protein vaccinations to elicit enhanced humoral and/or cellular immune responses. In particular, the immunostimulant constructs comprise anti- ***HER2*** antibodies fused to cytokines. In one example, an ant- ***HER2*** antibody-GM-CSF construct was shown to elicit an enhanced antitumor response.

AB . . . as adjuvants for antigenic protein vaccinations to elicit enhanced humoral and/or cellular immune responses. In particular, the immunostimulant constructs comprise anti- ***HER2*** antibodies fused to cytokines. In one example, an ant- ***HER2*** antibody-GM-CSF construct was shown to elicit an enhanced antitumor response.

IT Prion proteins
Staphylococcal ***protein*** ***A***
Tumor antigens
neu (receptor)
RL: BSU (Biological study, unclassified); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(adjuvant activity of antibody-immunostimulant fusion proteins targeted to)

L14 ANSWER 16 OF 31 CAPLUS COPYRIGHT 2009 ACS on STN
AN 2007:1186922 CAPLUS <<LOGINID::20090428>>
DN 149:362696
TI 99mTc-chelator engineering to improve tumour targeting properties of a ***HER2*** -specific Affibody molecule
AU Engfeldt, Torun; Tran, Thuy; Orlova, Anna; Widstroem, Charles; Feldwisch, Joachim; Abrahmsen, Lars; Wennborg, Anders; Karlstroem, Amelie Eriksson; Tolmachev, Vladimir
CS School of Biotechnology, Royal Institute of Technology, Stockholm, Swed.
SO European Journal of Nuclear Medicine and Molecular Imaging (2007), 34(11), 1843-1853
CODEN: EJNMA6; ISSN: 1619-7070
PB Springer
DT Journal
LA English

AB Purpose Monitoring ***HER2*** expression is crucial for selection of breast cancer patients amenable to ***HER2*** -targeting therapy. The Affibody mol. ZHER2:342 binds to ***HER2*** with picomolar affinity and enables specific imaging of ***HER2*** expression. Previously, ZHER2:342 with the addnl. N-terminal mercaptoacetyl-glycyl-glycyl-glycyl (maGGG) sequence was labeled with 99mTc and demonstrated specific targeting of ***HER2*** -expressing xenografts. However, hepatobiliary excretion caused high radioactivity accumulation in the abdomen. We investigated whether the biodistribution of ZHER2:342 can be improved by substituting glycyl residues in the chelating sequence with more hydrophilic seryl residues. Methods The Affibody mol. ZHER2:342, carrying the chelators mercaptoacetyl-glycyl-seryl-glycyl (maGSG), mercaptoacetyl-glycyl-D-seryl-glycyl [maG(D-S)G] and mercaptoacetyl-seryl-seryl-seryl (maSSS), were prepd. by peptide synthesis and labeled with 99mTc. The differences in the excretion pathways were evaluated in normal mice. The tumor targeting capacity of 99mTc-maSSS-ZHER2:342 was studied in nude mice bearing SKOV-3 xenografts and compared with the capacity of radioiodinated ZHER2:342. Results A shift towards renal excretion was obtained when glycine was substituted with serine in the chelating sequence. The radioactivity in the

gastrointestinal tract was reduced threefold for the maSSS conjugate in comparison with the maGGG conjugate 4 h post injection (p.i.). The tumor uptake of 99mTc-maSSS-ZHER2:342 was 11.5 .+-. 0.5% IA/g 4 h p.i., and the tumor-to-blood ratio was 76. The pharmacokinetics and uptake characteristics of technetium-labeled ZHER2:342 were better than those of radioiodinated ZHER2:342. Conclusion The introduction of serine residues in the chelator results in better tumor imaging properties of the Affibody mol. ZHER2:342 compared with glycyl-contg. chelators and is favorable for imaging of tumors and metastases in the abdominal area.

RE.CNT 35 THERE ARE 35 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

TI 99mTc-chelator engineering to improve tumour targeting properties of a ***HER2*** -specific Affibody molecule

AB Purpose Monitoring ***HER2*** expression is crucial for selection of breast cancer patients amenable to ***HER2*** -targeting therapy. The Affibody mol. ZHER2:342 binds to ***HER2*** with picomolar affinity and enables specific imaging of ***HER2*** expression. Previously, ZHER2:342 with the addnl. N-terminal mercaptoacetyl-glycyl-glycyl-glycyl (maGGG) sequence was labeled with 99mTc and demonstrated specific targeting of ***HER2*** -expressing xenografts. However, hepatobiliary excretion caused high radioactivity accumulation in the abdomen. We investigated whether the biodistribution of ZHER2:342 can be. . .

IT Animal cell line
(SKOV-3; 99mTc-chelator engineering to improve tumor targeting properties of ***HER2*** -specific Affibody mol.)

IT ***Staphylococcal*** ***protein*** ***A***
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(Z-domain synthetic homologues; 99mTc-chelator engineering to improve tumor targeting properties of ***HER2*** -specific Affibody mol.)

IT Drug delivery systems
(targeted; 99mTc-chelator engineering to improve tumor targeting properties of ***HER2*** -specific Affibody mol.)

IT Biological transport
(uptake; 99mTc-chelator engineering to improve tumor targeting properties of ***HER2*** -specific Affibody mol.)

IT Animal organ
Antitumor agents
Blood
Bone
Cecum
Chelating agents
Chirality
Human
Intestine
Kidney
Liver
Lung
Muscle
Neoplasm
Pharmacokinetics
Salivary gland
Spleen
Stability
Stomach
Thyroid gland
Urine
(99mTc-chelator engineering to improve tumor targeting properties of

HER2 -specific Affibody mol.)
IT neu (receptor)
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(99mTc-chelator engineering to improve tumor targeting properties of ***HER2*** -specific Affibody mol.)
IT 14133-76-7, Technetium 99, reactions
RL: RCT (Reactant); RACT (Reactant or reagent)
(Affibody chelator labeling by metastable; 99mTc-chelator engineering to improve tumor targeting properties of ***HER2*** -specific Affibody mol.)
IT 312-84-5, D-Serine
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(glycine substitution by; 99mTc-chelator engineering to improve tumor targeting properties of ***HER2*** -specific Affibody mol.)
IT 56-40-6, Glycine, biological studies
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(serine substitution of; 99mTc-chelator engineering to improve tumor targeting properties of ***HER2*** -specific Affibody mol.)
IT 66516-09-4
RL: PKT (Pharmacokinetics); BIOL (Biological study)
(99mTc-chelator engineering to improve tumor targeting properties of ***HER2*** -specific Affibody mol.)
IT 1056015-88-3DP, technetium 99m-labeled 1056015-89-4DP, technetium 99m-labeled 1056015-90-7DP, technetium 99m-labeled
RL: PKT (Pharmacokinetics); PRP (Properties); RCT (Reactant); SPN (Synthetic preparation); BIOL (Biological study); PREP (Preparation); RACT (Reactant or reagent)
(99mTc-chelator engineering to improve tumor targeting properties of ***HER2*** -specific Affibody mol.)

L14 ANSWER 17 OF 31 BIOSIS COPYRIGHT (c) 2009 The Thomson Corporation on STN DUPLICATE 3
AN 2008:30542 BIOSIS <<LOGINID::20090428>>
DN PREV200800019167
TI Affibody molecules for molecular imaging and therapy for cancer.
AU Orlova, Anna [Reprint Author]; Feldwisch, Joachim; Abrahmsen, Lars; Tolmachev, Vladimir
CS Uppsala Univ, Rudbeck Lab, Dept Oncol Radiol and Clin Immunol, Rudbeck Lab,Unit Biomed Radiat Sci, Dag Hammarskjolds 20, S-75185 Uppsala, Sweden anna.orlova@bms.uu.se
SO Cancer Biotherapy & Radiopharmaceuticals, (OCT 2007) Vol. 22, No. 5, pp. 573-584.
ISSN: 1084-9785.
DT Article
General Review; (Literature Review)
LA English
ED Entered STN: 19 Dec 2007
Last Updated on STN: 31 Jul 2008
AB Affibody molecules are scaffold proteins, having a common frame of amino acids determining the overall fold or tertiary structure, but with each member characterized by a unique amino acid composition in an exposed binding surface determining binding specificity and affinity for a certain target. Affibody molecules represent a new class of affinity proteins based on a 58-amino acid residue protein domain, derived from one of the IgG binding domains of ***staphylococcal*** ***protein*** ***A*** . They combine small size (similar to 65 kDa) with high affinity

and specificity. Affibody molecules with nanomolar affinities were selected from an initial library (3 x 10(9) members) and, after affinity maturation, picomolar binders were obtained. The small size and simple structure of affibody molecules allow their production by chemical synthesis with homogeneous site-specific incorporation of moieties for further labeling using a wide range of labeling chemistries. The robustness and the refolding properties of affibody molecules make them amenable to labeling conditions that denature most proteins, including incubation at pH 11 at 60 degrees C for up to 60 minutes. Affibody molecules meet the requirements which are key for successful clinical use as imaging agents: high-affinity binding to the chosen target; short plasma half-life time; rapid renal clearance for nonbound drug substance and, high, continuously increasing tumor-to-organ ratios, resulting in high-contrast in vivo images shortly after injection of the diagnostic agent.

AB. . . of affinity proteins based on a 58-amino acid residue protein domain, derived from one of the IgG binding domains of ***staphylococcal*** ***protein*** ***A*** . They combine small size (similar to 65 kDa) with high affinity and specificity. Affibody molecules with nanomolar affinities were selected. . .

IT . . .
lymphatics

IT Chemicals & Biochemicals
immunoglobulin G [IgG]; cyclophosphamide: antineoplastic-drug; methotrexate: antineoplastic-drug, enzyme inhibitor-drug; epidermal growth factor receptor [EGFR]: expression; ***HER2*** ; fluorouracil: antineoplastic-drug; HER1; ***staphylococcal*** ***protein*** ***A*** ; HER3: expression; affibody molecule

L14 ANSWER 18 OF 31 CAPLUS COPYRIGHT 2009 ACS on STN DUPLICATE 4
AN 2007:328411 CAPLUS <<LOGINID::20090428>>
DN 147:85823
TI Affibody molecules: potential for in vivo imaging of molecular targets for cancer therapy
AU Tolmachev, Vladimir; Orlova, Anna; Nilsson, Fredrik Y.; Feldwisch, Joachim; Wennborg, Anders; Abrahmsen, Lars
CS Affibody AB, Bromma, SE-161 02, Swed.
SO Expert Opinion on Biological Therapy (2007), 7(4), 555-568
CODEN: EOBTA2; ISSN: 1471-2598
PB Informa Healthcare
DT Journal; General Review
LA English
AB A review. Targeting radionuclide imaging of tumor-assocd. antigens may help to select patients who will benefit from a particular biol. therapy. Affibody mols. are a novel class of small (.apprx. 7 kDa) phage display-selected affinity proteins, based on the B-domain scaffold of ***staphylococcal*** ***protein*** ***A*** . A large library (3 .times. 109 variants) has enabled selection of high-affinity (up to 22 pM) binders for a variety of tumor-assocd. antigens. The small size of Affibody mols. provides rapid tumor localization and fast clearance from nonspecific compartments. Preclin. studies have demonstrated the potential of Affibody mols. for specific and high-contrast radionuclide imaging of ***HER2*** in vivo, and pilot clin. data using indium-111 and gallium-68 labeled anti- ***HER2*** Affibody tracer have confirmed its utility for radionuclide imaging in cancer patients.
RE.CNT 106 THERE ARE 106 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

AB . . . mols. are a novel class of small (.apprx. 7 kDa) phage display-selected affinity proteins, based on the B-domain scaffold of ***staphylococcal*** ***protein*** ***A*** . A large library (3 .times. 109 variants) has enabled selection of high-affinity (up to 22 pM) binders for a variety. . . clearance from nonspecific compartments. Preclin. studies have demonstrated the potential of Affibody mols. for specific and high-contrast radionuclide imaging of ***HER2*** in vivo, and pilot clin. data using indium-111 and gallium-68 labeled anti-***HER2*** Affibody tracer have confirmed its utility for radionuclide imaging in cancer patients.
ST review Affibody imaging ***HER2*** tumor assocd antigen cancer

L14 ANSWER 19 OF 31 MEDLINE on STN
AN 2007195534 MEDLINE <<LOGINID::20090428>>
DN PubMed ID: 17330952
TI In vivo evaluation of cysteine-based chelators for attachment of 99mTc to tumor-targeting Affibody molecules.
AU Tran Thuy; Engfeldt Torun; Orlova Anna; Widstrom Charles; Bruskin Alexander; Tolmachev Vladimir; Karlstrom Amelie Eriksson
CS Division of Biomedical Radiation Sciences, Rudbeck Laboratory, Uppsala University, Sweden.. thuy.tran@bms.uu.se
SO Bioconjugate chemistry, (2007 Mar-Apr) Vol. 18, No. 2, pp. 549-58. Electronic Publication: 2007-03-02.
Journal code: 9010319. ISSN: 1043-1802.
CY United States
DT (EVALUATION STUDIES)
Journal; Article; (JOURNAL ARTICLE)
(RESEARCH SUPPORT, NON-U.S. GOV'T)
LA English
FS Priority Journals
EM 200705
ED Entered STN: 3 Apr 2007
Last Updated on STN: 4 May 2007
Entered Medline: 3 May 2007
AB Affibody molecules present a new class of affinity proteins, which utilizes a scaffold based on a 58-amino acid domain derived from protein A. The small (7 kDa) Affibody molecule can be selected to bind to cell-surface targets with high affinity. An Affibody molecule (ZHER2:342) with a dissociation constant (Kd) of 22 pM for binding to the ***HER2*** receptor has been reported earlier. Preclinical and pilot clinical studies have demonstrated the utility of radiolabeled ZHER2:342 in imaging of ***HER2*** -expressing tumors. The small size and cysteine-free structure of Affibody molecules enable complete peptide synthesis and direct incorporation of radionuclide chelators. The goal of this study was to evaluate if incorporation of the natural peptide sequences cysteine-diglycine (CGG) and cysteine-triglycine (CGGG) sequences would enable labeling of Affibody molecules with 99mTc. In a model monomeric form, the chelating sequences were incorporated by peptide synthesis. The ***HER2*** -binding affinity was 280 and 250 pM for CGG-ZHER2:342 and CGGG-ZHER2:342, respectively. Conjugates were directly labeled with 99mTc with 90% efficiency and preserved the capacity to bind specifically to ***HER2*** -expressing cells. The biodistribution in normal mice showed a rapid clearance from the blood and the majority of organs (except kidneys). In the mice bearing SKOV-3 xenografts, tumor uptake of 99mTc-CGG-ZHER2:342 was ***HER2*** -specific and a tumor-to-blood ratio of 9.2 was obtained at 6 h postinjection. Gamma-camera imaging with 99mTc-CGG-ZHER2:342 clearly visualized tumors at 6 h postinjection. The

results show that the use of a cysteine-based chelator enables 99mTc-labeling of Affibody molecules for imaging.

AB . . . targets with high affinity. An Affibody molecule (ZHER2:342) with a dissociation constant (Kd) of 22 pM for binding to the ***HER2*** receptor has been reported earlier. Preclinical and pilot clinical studies have demonstrated the utility of radiolabeled ZHER2:342 in imaging of ***HER2*** -expressing tumors. The small size and cysteine-free structure of Affibody molecules enable complete peptide synthesis and direct incorporation of radionuclide chelators.. . . labeling of Affibody molecules with 99mTc. In a model monomeric form, the chelating sequences were incorporated by peptide synthesis. The ***HER2*** -binding affinity was 280 and 250 pM for CGG-ZHER2:342 and CGGG-ZHER2:342, respectively. Conjugates were directly labeled with 99mTc with 90% efficiency and preserved the capacity to bind specifically to ***HER2*** -expressing cells. The biodistribution in normal mice showed a rapid clearance from the blood and the majority of organs (except kidneys). In the mice bearing SKOV-3 xenografts, tumor uptake of 99mTc-CGG-ZHER2:342 was ***HER2*** -specific and a tumor-to-blood ratio of 9.2 was obtained at 6 h postinjection. Gamma-camera imaging with 99mTc-CGG-ZHER2:342 clearly visualized tumors at. . .

CT . . .
*Ovarian Neoplasms: RI, radionuclide imaging
Radiopharmaceuticals: DU, diagnostic use
Radiopharmaceuticals: PK, pharmacokinetics
Receptor, erbB-2: IM, immunology
*Receptor, erbB-2: ME, metabolism
****Staphylococcal Protein A: CH, chemistry***
*** Staphylococcal Protein A: ME, metabolism***
Tissue Distribution
Xenograft Model Antitumor Assays

CN 0 (Binding Sites, Antibody); 0 (Chelating Agents); 0 (Iodine Radioisotopes); 0 (Oligopeptides); 0 (Radiopharmaceuticals); 0 (***Staphylococcal*** ***Protein*** ***A***); EC 2.7.1.112 (Receptor, erbB-2)

L14 ANSWER 20 OF 31 SCISEARCH COPYRIGHT (c) 2009 The Thomson Corporation on STN

AN 2007:503517 SCISEARCH <<LOGINID::20090428>>

GA The Genuine Article (R) Number: 156LV

TI Adenovirus 5 vector genetically re-targeted by an affibody molecule with specificity for tumor antigen ***HER2*** /neu

AU Lindholm, L. (Reprint)

CS Got Gene AB, Kyviksvagen 18, SE-42930 Kullavik, Sweden (Reprint)

AU Magnusson, M. K.; Henning, P.; Myhre, S.; Wikman, M.; Uil, T. G.; Friedman, M.; Andersson, K. M. E.; Hong, S. S.; Hoeben, R. C.; Habib, N. A.; Stahl, S.; Boulanger, P.

CS Got Gene AB, SE-42930 Kullavik, Sweden; Univ Gothenburg, Inst Biomed, Dept Microbiol & Immunol, Gothenburg, Sweden; Albanova Univ Ctr, Kungl Teckn Hogskolan, Dept Biotechnol, Stockholm, Sweden; Leiden Univ, Med Ctr, Dept Mol Cell Biol, Leiden, Netherlands; Univ Lyon 1, Lab Virol & Pathogenese Virale, CNRS, UMR 5537, Fac Med RTH Laennec, F-69365 Lyon, France; Univ London Imperial Coll Sci Technol & Med, Fac Med, Dept Surg Oncol & Technol, London, England
E-mail: leif.lindholm@gotagene.se

CYA Sweden; Netherlands; France; England

SO CANCER GENE THERAPY, (MAY 2007) Vol. 14, No. 5, pp. 468-479.
ISSN: 0929-1903.

PB NATURE PUBLISHING GROUP, MACMILLAN BUILDING, 4 CRINAN ST, LONDON N1 9XW, ENGLAND.
DT Article; Journal
LA English
REC Reference Count: 54
ED Entered STN: 31 May 2007
Last Updated on STN: 31 May 2007
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
AB In order to use adenovirus (Ad) type 5 (Ad5) for cancer gene therapy, Ad needs to be de-targeted from its native receptors and re-targeted to a tumor antigen. A limiting factor for this has been to find a ligand that (i) binds a relevant target, (ii) is able to fold correctly in the reducing environment of the cytoplasm and (iii) when incorporated at an optimal position on the virion results in a virus with a low physical particle to plaque-forming units ratio to diminish the viral load to be administered to a future patient. Here, we present a solution to these problems by producing a genetically re-targeted Ad with a tandem repeat of the ***HER2*** /neu reactive Affibody molecule (ZH) in the HI-loop of a Coxsackie B virus and Ad receptor (CAR) binding ablated fiber genetically modified to contain sequences for flexible linkers between the ZH and the knob sequences. ZH is an Affibody molecule specific for the extracellular domain of ***human*** ***epidermal*** ***growth*** ***factor*** ***receptor*** ***2*** (***HER2*** /neu) that is overexpressed in inter alia breast and ovarian carcinomas. The virus presented here exhibits near wild-type growth characteristics, infects cells via ***HER2*** /neu instead of CAR and represents an important step toward the development of genetically re-targeted adenoviruses with clinical relevance.
TI Adenovirus 5 vector genetically re-targeted by an affibody molecule with specificity for tumor antigen ***HER2*** /neu
AB . . . Here, we present a solution to these problems by producing a genetically re-targeted Ad with a tandem repeat of the ***HER2*** /neu reactive Affibody molecule (ZH) in the HI-loop of a Coxsackie B virus and Ad receptor (CAR) binding ablated fiber genetically. . . flexible linkers between the ZH and the knob sequences. ZH is an Affibody molecule specific for the extracellular domain of ***human*** ***epidermal*** ***growth*** ***factor*** ***receptor*** ***2*** (***HER2*** /neu) that is overexpressed in inter alia breast and ovarian carcinomas. The virus presented here exhibits near wild-type growth characteristics, infects cells via ***HER2*** /neu instead of CAR and represents an important step toward the development of genetically re-targeted adenoviruses with clinical relevance.
ST Author Keywords: adenovirus; Affibody molecules; re-targeting; ***HER2*** /neu; fiber; HI-loop
STP KeyWords Plus (R): ***STAPHYLOCOCCAL*** ***PROTEIN*** - ***A*** ; FIBER PROTEIN; GENE-TRANSFER; INTRACELLULAR TRAFFICKING; RECEPTOR ANTIBODIES; CELLULAR RECEPTOR; KNOBLESS FIBERS; BINDING SITE; CANCER; DOMAIN

L14 ANSWER 21 OF 31 BIOSIS COPYRIGHT (c) 2009 The Thomson Corporation on STN
AN 2006:671264 BIOSIS <<LOGINID::20090428>>
DN PREV200600678305
TI Synthesis of technetium-chelating affibody molecules for diagnostic imaging of ***HER2*** -expressing tumours.
AU Engfeldt, T. [Reprint Author]; Tran, T.; Orlova, A.; Widstrom, C.;

Feldwisch, J.; Abrahamsen, L.; Wennborg, A.; Karlstrom, A. Eriksson; Tolmachev, V.
CS Royal Inst Technol, Sch Biotechnol, Stockholm, Sweden
SO Journal of Peptide Science, (2006) Vol. 12, No. Suppl. S, pp. 229. Meeting Info.: 29th European Peptide Symposium. Gdansk, POLAND. September 03 -08, 2006.
ISSN: 1075-2617.
DT Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LA English
ED Entered STN: 6 Dec 2006
Last Updated on STN: 6 Dec 2006
TI Synthesis of technetium-chelating affibody molecules for diagnostic imaging of ***HER2*** -expressing tumours.
IT . . .
IT Parts, Structures, & Systems of Organisms
blood: blood and lymphatics; abdomen; SKOV-3 ovarian carcinoma cell
IT Chemicals & Biochemicals
HER2 ; cell surface receptor; ***staphylococcal*** ***protein*** ***A*** ; affibody molecule; 99mTc-chelating sequence; mercaptoacetyltriglycyl [MAG3]; mercaptoacetyltriserinyl [MAS3]
IT . . .
microscopy techniques; molecular imaging: laboratory techniques, imaging and microscopy techniques
IT Miscellaneous Descriptors
biodistribution; chemical synthesis; hepatobiliary excretion; Fmoc/tBu chemistry; ***HER2*** -expressing tumor

L14 ANSWER 22 OF 31 BIOSIS COPYRIGHT (c) 2009 The Thomson Corporation on STN
AN 2006:366082 BIOSIS <<LOGINID::20090428>>
DN PREV200600371017
TI AlphaB-crystallin: A novel marker for metaplastic and basal-like breast cancers.
AU Sitterding, S. M. [Reprint Author]; Wiseman, W. R.; Schiller, C. L.; Watkin, W. G.; Luan, C.; Wiley, E. L.; Moyano, J. V.; Cryns, V. L.; Diaz, L. K.
CS Northwestern Univ, Chicago, IL 60611 USA
SO Laboratory Investigation, (JAN 2006) Vol. 86, No. Suppl. 1, pp. 42A-43A. Meeting Info.: 95th Annual Meeting of the United-States-and-Canadian-Academy-of-Pathology. Atlanta, GA, USA. February 11 -17, 2006. US & Canadian Acad Pathol.
CODEN: LAINAW. ISSN: 0023-6837.
DT Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LA English
ED Entered STN: 26 Jul 2006
Last Updated on STN: 26 Jul 2006
IT . . .
tumor: neoplastic disease, reproductive system disease/female
IT Diseases
metaplastic breast tumor: neoplastic disease, reproductive system disease/female
IT Chemicals & Biochemicals
HER2 : expression; cytokeratin 5/6: expression; HER1: expression; alphaB-crystallin: oncoprotein, small heat shock protein,

expression
IT Methods & Equipment
gene expression profiling: laboratory techniques, genetic techniques; immunohistochemical staining: laboratory techniques, histology and cytology techniques, immunologic techniques; ***SPA*** -222
antibody: medical equipment, Stressgen Biotechnologies
IT Miscellaneous Descriptors
immunophenotype

L14 ANSWER 23 OF 31 CAPLUS COPYRIGHT 2009 ACS on STN
AN 2005:34770 CAPLUS <<LOGINID::20090428>>
DN 142:109117
TI Her-2 receptor-binding derivatives of ***Staphylococcal*** ***protein*** ***A*** for use in diagnosis and therapy of cancer
IN Carlsson, Joergen; Stahl, Stefan; Eriksson, Tove; Gunneriusson, Elin; Nilsson, Fredrik
PA Affibody AB, Swed.
SO PCT Int. Appl., 116 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 2005003156	A1	20050113	WO 2004-SE1049	20040630
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW				
RW: BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
AU 2004253835	A1	20050113	AU 2004-253835	20040630
AU 2004253835	B2	20090129		
CA 2531238	A1	20050113	CA 2004-2531238	20040630
EP 1641818	A1	20060405	EP 2004-749087	20040630
EP 1641818	B1	20081203		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, FI, RO, CY, TR, BG, CZ, EE, HU, PL, SK				
CN 1816563	A	20060809	CN 2004-80019059	20040630
JP 2007537700	T	20071227	JP 2006-518586	20040630
AT 416190	T	20081215	AT 2004-749087	20040630
IN 2005KN02544	A	20061013	IN 2005-KN2544	20051209
PRAI SE 2003-1987	A	20030704		
SE 2004-275	A	20040209		
WO 2004-SE1049	W	20040630		

AB Substitution derivs. of the Z domain of ***Staphylococcal*** ***protein*** ***A*** (***SPA***) with a strong, specific, binding affinity for ***HER2*** are described for use in the diagnosis and treatment of ***her2*** -dependent cancers. A gene for the protein and l expression vectors and host cells for manuf. of the protein are also described. Also provided is the use of such a polypeptide as a medicament, and as a targeting agent for directing substances conjugated

thereto to cells overexpressing ***HER2*** . The specificity of binding of the protein for the receptor allows its use in drug targeting with minimal side effects. Methods, and kits for performing the methods, are also provided, which methods and kits rely on the binding of the polypeptide to ***HER2*** . The proteins were identified in combinatorial libraries by panning. The protein manufd. in Escherichia coli bound to ***HER2*** -bearing SKBR-3 cells. The protein was well-tolerated by injection when given to nude mice bearing SKOV-3 cell implants. The protein was accumulated rapidly in SKOV-3 cells.

RE.CNT 6 THERE ARE 6 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

TI Her-2 receptor-binding derivatives of ***Staphylococcal*** ***protein*** ***A*** for use in diagnosis and therapy of cancer

AB Substitution derivs. of the Z domain of ***Staphylococcal*** ***protein*** ***A*** (***SPA***) with a strong, specific, binding affinity for ***HER2*** are described for use in the diagnosis and treatment of ***her2*** -dependent cancers. A gene for the protein and l expression vectors and host cells for manuf. of the protein are also. . . of such a polypeptide as a medicament, and as a targeting agent for directing substances conjugated thereto to cells overexpressing ***HER2*** . The specificity of binding of the protein for the receptor allows its use in drug targeting with minimal side effects.. . . kits for performing the methods, are also provided, which methods and kits rely on the binding of the polypeptide to ***HER2*** . The proteins were identified in combinatorial libraries by panning. The protein manufd. in Escherichia coli bound to ***HER2*** -bearing SKBR-3 cells. The protein was well-tolerated by injection when given to nude mice bearing SKOV-3 cell implants. The protein was. . .

ST ***HER2*** binding Staphylococcus protein cancer diagnosis therapy

IT Protein engineering
(of protein binding by ***Staphylococcal*** ***protein*** ***A*** ; her-2 receptor-binding derivs. of Staphylococcal protein for
use in diagnosis and therapy of cancer)

IT Albumins, biological studies
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(serum, protein A derivs. binding ***her2*** receptors and; her-2 receptor-binding derivs. of Staphylococcal protein for use in diagnosis and therapy of cancer)

IT Mutation
(substitution, effects on protein binding by ***Staphylococcal*** ***protein*** ***A*** ; her-2 receptor-binding derivs. of Staphylococcal protein for use in diagnosis and therapy of cancer)

IT 823578-05-8 823578-06-9
RL: PRP (Properties)
(unclaimed sequence; her-2 receptor-binding derivs. of ***Staphylococcal*** ***protein*** ***A*** for use in diagnosis and therapy of cancer)

L14 ANSWER 24 OF 31 MEDLINE on STN
AN 2005610503 MEDLINE <<LOGINID::20090428>>
DN PubMed ID: 16287254
TI Evaluation of ((4-hydroxyphenyl)ethyl)maleimide for site-specific radiobromination of anti- ***HER2*** affibody.
AU Mume Eskender; Orlova Anna; Larsson Barbro; Nilsson Ann-Sofie; Nilsson Fredrik Y; Sjoberg Stefan; Tolmachev Vladimir
CS Department of Chemistry, Organic Chemistry, Uppsala University, Uppsala,

Sweden.
SO Bioconjugate chemistry, (2005 Nov-Dec) Vol. 16, No. 6, pp. 1547-55.
Journal code: 9010319. ISSN: 1043-1802.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
(RESEARCH SUPPORT, NON-U.S. GOV'T)
LA English
FS Priority Journals
EM 200601
ED Entered STN: 22 Nov 2005
Last Updated on STN: 24 Jan 2006
Entered Medline: 23 Jan 2006
AB Affibody molecules are a new class of small phage-display selected proteins using a scaffold domain of the bacterial receptor protein A. They can be selected for specific binding to a large variety of protein targets. An affibody molecule binding with high affinity to a tumor antigen ***HER2*** was recently developed for radionuclide diagnostics and therapy in vivo. The use of the positron-emitting nuclide (76)Br (T(1/2) = 16.2 h) could improve the sensitivity of detection of ***HER2*** -expressing tumors. A site-specific radiobromination of a cysteine-containing variant of the anti- ***HER2*** affibody, (Z(***HER2*** :4))(2)-Cys, using ((4-hydroxyphenyl)ethyl)maleimide (HPEM), was evaluated in this study. It was found that HPEM can be radiobrominated with an efficiency of 83 +/- 0.4% and thereafter coupled to freshly reduced affibody with a yield of 65.3 +/- 3.9%. A "one-pot" labeling enabled the radiochemical purity of the conjugate to exceed 97%. The label was stable against challenge with large excess of nonlabeled bromide and in a high molar strength solution. In vitro cell tests demonstrated that radiobrominated affibody binds specifically to the ***HER2*** -expressing cell-line, SK-OV-3. Biodistribution studies in nude mice bearing SK-OV-3 xenografts have shown tumor accumulation of 4.8 +/- 2.2% IA/g and good tumor-to-normal tissue ratios.
TI Evaluation of ((4-hydroxyphenyl)ethyl)maleimide for site-specific radiobromination of anti- ***HER2*** affibody.
AB . . . specific binding to a large variety of protein targets. An affibody molecule binding with high affinity to a tumor antigen ***HER2*** was recently developed for radionuclide diagnostics and therapy in vivo. The use of the positron-emitting nuclide (76)Br (T(1/2) = 16.2 h) could improve the sensitivity of detection of ***HER2*** -expressing tumors. A site-specific radiobromination of a cysteine-containing variant of the anti- ***HER2*** affibody, (Z(***HER2*** :4))(2)-Cys, using ((4-hydroxyphenyl)ethyl)maleimide (HPEM), was evaluated in this study. It was found that HPEM can be radiobrominated with an efficiency of. . . bromide and in a high molar strength solution. In vitro cell tests demonstrated that radiobrominated affibody binds specifically to the ***HER2*** -expressing cell-line, SK-OV-3. Biodistribution studies in nude mice bearing SK-OV-3 xenografts have shown tumor accumulation of 4.8 +/- 2.2% IA/g and. . .
CT . . . PK, pharmacokinetics
Protein Interaction Mapping
*Radiopharmaceuticals: CS, chemical synthesis
*Radiopharmaceuticals: PK, pharmacokinetics
Receptor, erbB-2: AN, analysis
*Receptor, erbB-2: ME, metabolism
*** Staphylococcal Protein A: CH, chemistry***
Tissue Distribution
Transplantation, Heterologous

CN 0 (Antigens, Neoplasm); 0 (Bromine Radioisotopes); 0 (Maleimides); 0 (Peptide Library); 0 (Peptides); 0 (Radiopharmaceuticals); 0 (***Staphylococcal*** ***Protein*** ***A***); EC 2.7.1.112 (Receptor, erbB-2)

L14 ANSWER 25 OF 31 LIFESCI COPYRIGHT 2009 CSA on STN
AN 2005:36890 LIFESCI <<LOGINID::20090428>>
TI Tumor cell targeted gene delivery by adenovirus 5 vectors carrying knobless fibers with antibody-binding domains
AU Henning, P.; Andersson, K.M.E.; Frykholm, K.; Ali, A.; Magnusson, M.K.; Nygren, P.-A.; Granio, O.; Hong, S.S.; Boulanger, P.; Lindholm, L.
CS Got-a-Gene AB, Stena Center 1B, Gothenburg SE 41292, Sweden
SO Gene Therapy [Gene Ther.], (20050200) vol. 12, no. 3, pp. 211-224. ISSN: 0969-7128.
DT Journal
FS G; W3
LA English
SL English
AB Most human carcinoma cell lines lack the high-affinity receptors for adenovirus serotype 5 (Ad5) at their surface and are nonpermissive to Ad5. We therefore tested the efficiency of retargeting Ad5 to alternative cellular receptors via immunoglobulin (Ig)-binding domains inserted at the extremity of short-shafted, knobless fibers. The two recombinant Ad5's constructed, Ad5/R7-Z sub(wt)-Z sub(wt) and Ad5/R7-C2-C2, carried tandem Ig-binding domains from ***Staphylococcal*** ***protein*** ***A*** (abbreviated Z sub(wt)) and from Streptococcal protein G (C2), respectively. Both viruses bound their specific Ig isotypes with the expected affinity. They transduced human carcinoma cells independently of the CAR pathway, via cell surface receptors targeted by specific monoclonal antibodies, that is, EGF-R on A549, HT29 and SW1116, HER-2/neu on SK-OV-3 and SK-BR-3, CA242 (epitope recognized by the monoclonal antibody C242) antigen on HT29 and SW1116, and PSMA (prostate-specific membrane antigen) expressed on HEK-293 cells, respectively. However, Colo201 and Colo205 cells were neither transduced by targeting CA242 or EGF-R nor were LNCaP cells transduced by targeting PSMA. Our results suggested that one given surface receptor could mediate transduction of certain cells but not others, indicating that factors and steps other than cell surface expression and virus-receptor interaction are additional determinants of Ad5-mediated transduction of tumor cells. Using penton base RGD mutants, we found that one of these limiting steps was virus endocytosis.
AB . . . extremity of short-shafted, knobless fibers. The two recombinant Ad5's constructed, Ad5/R7-Z sub(wt)-Z sub(wt) and Ad5/R7-C2-C2, carried tandem Ig-binding domains from ***Staphylococcal*** ***protein*** ***A*** (abbreviated Z sub(wt)) and from Streptococcal protein G (C2), respectively. Both viruses bound their specific Ig isotypes with the expected. . .
UT Immunoglobulins; Monoclonal antibodies; Carcinoma; Fibers; Cell surface; ***HER2*** protein; Gene therapy; Endocytosis; streptococcal protein G; protein A; Pentons; Tumor cell lines; Gene transfer; Calcium-sensing receptors; Neu protein; Expression. . .

L14 ANSWER 26 OF 31 BIOSIS COPYRIGHT (c) 2009 The Thomson Corporation on STN DUPLICATE 5
AN 2004:458754 BIOSIS <<LOGINID::20090428>>
DN PREV200400458361
TI Selection and characterization of ***HER2*** /neu-binding affibody

ligands.
AU Wikman, M.; Steffen, A.-C.; Gunneriusson, E.; Tolmachev, V.; Adams, G. P.; Carlsson, J.; Stahl, S. [Reprint Author]
CS AlbaNova Univ CtrDept Biotechnol, KTH, SE-10691, Stockholm, Sweden
stefans@biotech.kth.se
SO Protein Engineering Design & Selection, (May 2004) Vol. 17, No. 5, pp. 455-462. print.
ISSN: 1741-0126 (ISSN print).
DT Article
LA English
ED Entered STN: 24 Nov 2004
Last Updated on STN: 24 Nov 2004
AB Affibody(R) (affibody) ligands that are specific for the extracellular domain of ***human*** ***epidermal*** ***growth*** ***factor*** ***receptor*** ***2*** (***HER2*** /neu) have been selected by phage display technology from a combinatorial protein library based on the 58 amino acid residue ***staphylococcal*** ***protein*** ***A*** -derived Z domain. The predominant variants from the phage selection were produced in Escherichia coli, purified by affinity chromatography, and characterized by biosensor analyses. Two affibody variants were shown to selectively bind to the extracellular domain of ***HER2*** /neu (***HER2*** -ECD), but not to control proteins. One of the variants, denoted His6-ZHER2/neu:4, was demonstrated to bind with nanomolar affinity (apprx50 nM) to the ***HER2*** -ECD molecule at a different site than the monoclonal antibody trastuzumab. Furthermore, radiolabeled His6-ZHER2/neu:4 affibody showed specific binding to native ***HER2*** /neu, overexpressed on the SKBR-3 tumor cell line. Such affibody ligands might be considered in tumor targeting applications for radionuclide diagnostics and therapy of adenocarcinomas such as breast and ovarian cancers.
TI Selection and characterization of ***HER2*** /neu-binding affibody ligands.
AB Affibody(R) (affibody) ligands that are specific for the extracellular domain of ***human*** ***epidermal*** ***growth*** ***factor*** ***receptor*** ***2*** (***HER2*** /neu) have been selected by phage display technology from a combinatorial protein library based on the 58 amino acid residue ***staphylococcal*** ***protein*** ***A*** -derived Z domain. The predominant variants from the phage selection were produced in Escherichia coli, purified by affinity chromatography, and characterized by biosensor analyses. Two affibody variants were shown to selectively bind to the extracellular domain of ***HER2*** /neu (***HER2*** -ECD), but not to control proteins. One of the variants, denoted His6-ZHER2/neu:4, was demonstrated to bind with nanomolar affinity (apprx50 nM) to the ***HER2*** -ECD molecule at a different site than the monoclonal antibody trastuzumab. Furthermore, radiolabeled His6-ZHER2/neu:4 affibody showed specific binding to native ***HER2*** /neu, overexpressed on the SKBR-3 tumor cell line. Such affibody ligands might be considered in tumor targeting applications for radionuclide diagnostics. . .
IT . . .
(MeSH)
IT Diseases
ovarian cancer: neoplastic disease, reproductive system disease/female, diagnosis, therapy
Ovarian Neoplasms (MeSH)
IT Chemicals & Biochemicals
affibody ligands; ***human*** ***epidermal*** ***growth***

factor ***receptor*** ***2*** [***HER2*** /neu];
proteins; radionuclide; trastuzumab: monoclonal antibody

L14 ANSWER 27 OF 31 BIOSIS COPYRIGHT (c) 2009 The Thomson Corporation on STN DUPLICATE 6
AN 2003:281726 BIOSIS <<LOGINID::20090428>>
DN PREV200300281726
TI Antibody-mediated targeting of replication-competent retroviral vectors.
AU Tai, Chien-Kuo; Logg, Christopher R.; Park, Jinha M.; Anderson, W. French; Press, Michael F.; Kasahara, Noriyuki [Reprint Author]
CS Department of Medicine, Geffen School of Medicine, UCLA, 675 Charles E. Young Drive South, MacDonald Research Laboratories, MRL-1551, Los Angeles, CA, 90095, USA
nkasahara@mednet.ucla.edu
SO Human Gene Therapy, (May 20 2003) Vol. 14, No. 8, pp. 789-802. print.
ISSN: 1043-0342 (ISSN print).
DT Article
LA English
ED Entered STN: 19 Jun 2003
Last Updated on STN: 19 Jun 2003
AB Replication-competent murine leukemia virus (MLV) vectors can be engineered to achieve high efficiency gene transfer to solid tumors in vivo and tumor-restricted replication, however their safety can be further enhanced by redirecting tropism of the virus envelope. We have therefore tested the targeting capability and replicative stability of ecotropic and amphotropic replication-competent retrovirus (RCR) vectors containing two tandem repeats from the immunoglobulin G-binding domain of ***Staphylococcal*** ***protein*** ***A*** inserted into the proline-rich "hinge" region of the envelope, which enables modular use of antibodies of various specificities for vector targeting. The modified envelopes were efficiently expressed and incorporated into virions, were capable of capturing monoclonal anti- ***HER2*** antibodies, and mediated efficient binding of the virus-antibody complex to ***HER2*** -positive target cells. While infectivity was markedly reduced by pseudotyping with targeted envelopes alone, coexpression of wild-type envelope rescued efficient cellular entry. Both ecotropic and amphotropic RCR vector/anti- ***HER2*** antibody complexes achieved significant enhancement of transduction on murine target cells overexpressing ***HER2*** , which could be competed by preincubation with excess free antibodies. Interestingly, ***HER2*** -expressing human breast cancer cells did not show enhancement of transduction despite efficient antibody-mediated cell surface binding, suggesting that target cell-specific parameters markedly affect the efficiency of post-binding entry processes. Serial replication of targeted vectors resulted in selection of Z domain deletion variants, but reduction of the overall size of the vector genome enhanced its stability. Application of antibody-mediated targeting to the initial localization of replication-competent virus vectors to tumor sites will thus require optimized target selection and vector design.
AB. . . replicative stability of ecotropic and amphotropic replication-competent retrovirus (RCR) vectors containing two tandem repeats from the immunoglobulin G-binding domain of ***Staphylococcal*** ***protein*** ***A*** inserted into the proline-rich "hinge" region of the envelope, which enables modular use of antibodies of various specificities for vector targeting. The modified envelopes were efficiently expressed and incorporated into virions, were capable of capturing monoclonal anti- ***HER2*** antibodies, and mediated

efficient binding of the virus-antibody complex to ***HER2*** -positive target cells. While infectivity was markedly reduced by pseudotyping with targeted envelopes alone, coexpression of wild-type envelope rescued efficient cellular entry. Both ecotropic and amphotropic RCR vector/anti-***HER2*** antibody complexes achieved significant enhancement of transduction on murine target cells overexpressing ***HER2*** , which could be competed by preincubation with excess free antibodies. Interestingly, ***HER2*** -expressing human breast cancer cells did not show enhancement of transduction despite efficient antibody-mediated cell surface binding, suggesting that target cell-specific. . .

IT . . . Concepts
Immune System (Chemical Coordination and Homeostasis); Molecular Genetics (Biochemistry and Molecular Biophysics); Tumor Biology

IT Chemicals & Biochemicals
HER-2; ***Staphylococcal*** ***protein*** ***A*** : immunoglobulin G-binding domain; monoclonal anti- ***HER2*** antibodies; virus envelope: tropism

L14 ANSWER 28 OF 31 MEDLINE on STN
AN 2003572748 MEDLINE <<LOGINID::20090428>>
DN PubMed ID: 14644615
TI Vesicular stomatitis virus expressing a chimeric Sindbis glycoprotein containing an Fc antibody binding domain targets to ***Her2*** /neu overexpressing breast cancer cells.
AU Bergman Ira; Whitaker-Dowling Patricia; Gao Yanhua; Griffin Judith A; Watkins Simon C
CS Departments of Pediatric, University of Pittsburgh School of Medicine, Pittsburgh, PA 15213, USA.. ira.bergman@chp.edu
SO Virology, (2003 Nov 25) Vol. 316, No. 2, pp. 337-47.
Journal code: 0110674. ISSN: 0042-6822.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
(RESEARCH SUPPORT, NON-U.S. GOV'T)
(RESEARCH SUPPORT, U.S. GOV'T, NON-P.H.S.)
LA English
FS Priority Journals
EM 200401
ED Entered STN: 16 Dec 2003
Last Updated on STN: 7 Jan 2004
Entered Medline: 6 Jan 2004
AB Vesicular stomatitis virus (VSV) is a candidate for development for cancer therapy. It is an oncolytic virus that is safe in humans. Recombinant virus can be made directly from plasmid components. We attempted to create a virus that targeted specifically to breast cancer cells. Nonreplicating and replicating pseudotype VSV were created whose only surface glycoprotein (gp) was a Sindbis gp, called Sindbis-ZZ, modified to severely reduce its native binding function and to contain the Fc-binding domain of Staphylococcus aureus protein A. When titered on ***Her2*** /neu overexpressing SKBR3 human breast cancer cells, pseudotype VSV coated with Sindbis-ZZ had <1% the titer of pseudotype VSV coated with wild-type Sindbis gp. Titer was increased 50-fold when the Sindbis-ZZ pseudotype was conjugated with 4D5, a mouse monoclonal antibody directed against the ***Her2*** /neu receptor. Titers of antibody-conjugated virus were increased 36-fold on a second human breast cancer cell line, MCF7/H2, which expressed lower concentrations of ***Her2*** /neu receptor on the cell surface. At multiple concentrations of antibody, titers on SKBR3 cells were significantly greater when the virus was incubated with

Herceptin, an antibody with a human Fc, than with 4D5, a mouse antibody, reflecting the known higher affinity of the protein A Fc-binding domain for human Fc. Analysis of the protein composition of the pseudotype VSV found low expression of the modified Sindbis gp on the virus accounting, in part, for a viral titer that did not exceed 1.2 x 10(5)/ml. This work demonstrates the ability to easily create, directly from plasmid components, an oncolytic replicating VSV with a restricted host cell range.

TI Vesicular stomatitis virus expressing a chimeric Sindbis glycoprotein containing an Fc antibody binding domain targets to ***Her2*** /neu overexpressing breast cancer cells.

AB . . . severely reduce its native binding function and to contain the Fc-binding domain of Staphylococcus aureus protein A. When titered on ***Her2*** /neu overexpressing SKBR3 human breast cancer cells, pseudotype VSV coated with Sindbis-ZZ had <1% the titer of pseudotype VSV coated with. . . gp. Titer was increased 50-fold when the Sindbis-ZZ pseudotype was conjugated with 4D5, a mouse monoclonal antibody directed against the ***Her2*** /neu receptor. Titers of antibody-conjugated virus were increased 36-fold on a second human breast cancer cell line, MCF7/H2, which expressed lower concentrations of ***Her2*** /neu receptor on the cell surface. At multiple concentrations of antibody, titers on SKBR3 cells were significantly greater when the virus. . .

CT . . .

methods

Humans

Hydrogen-Ion Concentration

*Immunoglobulin Fc Fragments: GE, genetics

*Receptor, erbB-2: AI, antagonists & inhibitors

*Recombinant Fusion Proteins: GE, genetics

*** Staphylococcal Protein A: GE, genetics***

*Vesicular stomatitis Indiana virus: GE, genetics

*Viral Envelope Proteins: GE, genetics

CN 0 (Antibodies, Monoclonal); 0 (Immunoglobulin Fc Fragments); 0 (Recombinant Fusion Proteins); 0 (***Staphylococcal*** ***Protein*** ***A***); 0 (Viral Envelope Proteins); 0 (glycoprotein E2, Sindbis virus); EC 2.7.1.112 (Receptor, erbB-2)

L14 ANSWER 29 OF 31 BIOSIS COPYRIGHT (c) 2009 The Thomson Corporation on STN DUPLICATE 7

AN 2000:313922 BIOSIS <<LOGINID::20090428>>

DN PREV200000313922

TI Minimal catalytic domain of N-acetylglucosaminyltransferase V.

AU Korczak, Bozena [Reprint author]; Le, Thuyanh; Elowe, Sabine; Datti, Alessandro; Dennis, James W.

CS GlycoDesign Inc., 480 University Avenue, Suite 900, Toronto, Ontario, Canada

SO Glycobiology, (June, 2000) Vol. 10, No. 6, pp. 595-599. print. ISSN: 0959-6658.

DT Article

LA English

ED Entered STN: 26 Jul 2000
Last Updated on STN: 7 Jan 2002

AB UDP-GlcNAc: Manalpha1-6Manbeta-R beta1-6 N-acetylglucosaminyltransferase V (EC 2.4.1.155, GlcNAc-TV) is a Golgi enzyme that substitutes the trimannosyl core in the biosynthetic pathway for complex-type N-linked glycans. GlcNAc-TV activity is regulated by oncogenes frequently activated in cancer cells (ras, src, and ***her2*** /neu) and by

activators of T lymphocytes. Overexpression of GlcNAc-TV in epithelial cells results in morphological transformation, while tumor cell mutants selected for loss of GlcNAc-TV products show diminished malignant potential in mice. In this report, we have expressed and characterized a series of N- and C-terminal deletions of GlcNAc-TV. Portions of GlcNAc-TV sequence were fused at the N-terminal domain to IgG-binding domains of ***staphylococcal*** ***Protein*** ***A*** and expressed in CHOP cells. The secreted fusion proteins were purified by IgG Sepharose affinity chromatography and assayed for enzyme activities. The peptide sequence S213-740 of GlcNAc-TV was determined to be essential for the catalytic activity, the remaining amino acids comprising a 183 amino acid stem region, a 17 amino acid transmembrane domain and a 12 amino acid cytosolic moiety. Further deletion of 5 amino acids to produce peptide R218-740 reduced enzyme activity by 20-fold. Similar Km and Vmax values for donor and acceptor were observed for peptide S213-740, the minimal catalytic domain, and peptide Q39-740, which also included the stem region. Truncation of five amino acids from the C-terminus also resulted in a 20-fold loss of catalytic activity. Secondary structure predictions suggest a high frequency of turns in the stem region, and more contiguous stretches of alpha-helix found in the catalytic domain.

AB . . biosynthetic pathway for complex-type N-linked glycans. GlcNAc-TV activity is regulated by oncogenes frequently activated in cancer cells (ras, src, and ***her2*** /neu) and by activators of T lymphocytes. Overexpression of GlcNAc-TV in epithelial cells results in morphological transformation, while tumor cell mutants. . . N- and C-terminal deletions of GlcNAc-TV. Portions of GlcNAc-TV sequence were fused at the N-terminal domain to IgG-binding domains of ***staphylococcal*** ***Protein*** ***A*** and expressed in CHOP cells. The secreted fusion proteins were purified by IgG Sepharose affinity chromatography and assayed for enzyme. . .

L14 ANSWER 30 OF 31 BIOSIS COPYRIGHT (c) 2009 The Thomson Corporation on STN DUPLICATE 8

AN 2000:87883 BIOSIS <<LOGINID::20090428>>

DN PREV200000087883

TI Characterization of the binding interface between the E-domain of ***Staphylococcal*** ***protein*** ***A*** and an antibody Fv-fragment.

AU Meininger, David P.; Rance, Mark; Starovasnik, Melissa A.; Fairbrother, Wayne J. [Reprint author]; Skelton, Nicholas J. [Reprint author]

CS Department of Protein Engineering, Genentech, Inc., One DNA Way, South San Francisco, CA, 94080, USA

SO Biochemistry, (Jan. 11, 2000) Vol. 39, No. 1, pp. 26-36. print.
CODEN: BICHAW. ISSN: 0006-2960.

DT Article

LA English

ED Entered STN: 10 Mar 2000
Last Updated on STN: 3 Jan 2002

AB ***Staphylococcal*** ***protein*** ***A*** (***SpA***) is a cell-surface component of Staphylococcus aureus. In addition to the well-characterized interaction between ***SpA*** and the Fc-region of human IgG, an alternative binding interaction between ***SpA*** and the Fab-region of immunoglobulin domains encoded by the VH3 gene family has been described. To characterize structurally the interface formed by ***SpA*** repeats and type-3 VH-domains, we have studied the 32-kDa complex formed between an E-domain mutant (EZ4) and the Fv-fragment of the

humanized anti- ***HER2*** antibody (Hu4D5-8) using heteronuclear NMR spectroscopy. Protocols were established for efficient incorporation of 15N, 13C, and 2H into EZ4 and the VH- and VL-domains of the Fv, allowing backbone resonances to be assigned sequentially for EZ4 and the VH-domain in both free and complexed states, Broadening of certain VH-resonances in the free and bound Fv-fragment suggests microsecond to millisecond time-scale motion in CDR3. Residues experiencing significant chemical shift changes of backbone 1HN, 15N, and 13CO resonances upon complex formation delineate contiguous surfaces on EZ4 and the VH-domain that define the binding interfaces of the two proteins. The interaction surfaces identified by chemical shift mapping are comprised of predominantly hydrophilic residues. This is in contrast to the ***SpA*** -Fc interface which is predominantly hydrophobic in nature. Further analysis of the surface properties suggests a probable binding orientation for ***SpA*** - and VH3-domains.

TI Characterization of the binding interface between the E-domain of ***Staphylococcal*** ***protein*** ***A*** and an antibody Fv-fragment.

AB ***Staphylococcal*** ***protein*** ***A*** (***SpA***) is a cell-surface component of Staphylococcus aureus. In addition to the well-characterized interaction between ***SpA*** and the Fc-region of human IgG, an alternative binding interaction between ***SpA*** and the Fab-region of immunoglobulin domains encoded by the VH3 gene family has been described. To characterize structurally the interface formed by ***SpA*** repeats and type-3 VH-domains, we have studied the 32-kDa complex formed between an E-domain mutant (EZ4) and the Fv-fragment of the humanized anti- ***HER2*** antibody (Hu4D5-8) using heteronuclear NMR spectroscopy. Protocols were established for efficient incorporation of 15N, 13C, and 2H into EZ4 and. . . The interaction surfaces identified by chemical shift mapping are comprised of predominantly hydrophilic residues. This is in contrast to the ***SpA*** -Fc interface which is predominantly hydrophobic in nature. Further analysis of the surface properties suggests a probable binding orientation for ***SpA*** - and VH3-domains.

IT Major Concepts
Biochemistry and Molecular Biophysics; Methods and Techniques

IT Chemicals & Biochemicals
EZ4: E-domain mutant; anti- ***HER2*** : antibody; antibody Fv-fragment; carbon-13: label; deuterium: label; nitrogen-15: label; ***staphylococcal*** ***protein*** ***A*** : characterization, purification

L14 ANSWER 31 OF 31 CAPLUS COPYRIGHT 2009 ACS on STN DUPLICATE 9
AN 1992:405736 CAPLUS <<LOGINID::20090428>>
DN 117:5736
OREF 117:1195a,1198a
TI Antigen binding thermodynamics and antiproliferative effects of chimeric and humanized anti-p185HER2 antibody Fab fragments
AU Kelley, Robert F.; O'Connell, Mark P.; Carter, Paul; Presta, Leonard; Eigenbrot, Charles; Covarrubias, Michael; Snedecor, Brad; Bourell, James H.; Vetterlein, David
CS Dep. Protein Eng., Genentech, Inc., South San Francisco, CA, 94080, USA
SO Biochemistry (1992), 31(24), 5434-41
CODEN: BICHAW; ISSN: 0006-2960
DT Journal
LA English

AB The murine monoclonal antibody 4D5 (anti-p185HER2) inhibits the proliferation of human tumor cells overexpressing p185HER2 in vitro and has been humanized (Carter, et al., 1991) for use in human cancer therapy. The antigen binding thermodn. and the antiproliferative activities were detd. of chimeric 4D5 Fab (ch4D5 Fab) fragment and a series of 8 humanized Fab (hu4D5 Fab) fragments differing by amino acid substitutions in the framework regions of the variable domains. Fab fragments were expressed by secretion from Escherichia coli and purified from fermn. supernatants by using affinity chromatog. on immobilized streptococcal protein G or ***staphylococcal*** ***protein*** ***A*** for ch4D5 and hu4D5, resp. CD spectroscopy indicates correct folding of the E. coli produced Fab, and scanning calorimetry shows a greater stability for hu4D5, (Tm = 82.degree.) as compared with ch4D5 Fab (Tm = 72.degree.). KD Values for binding to the extracellular domain (ECD) of p185HER2 were detd. by using a RIA; the .DELTA.H and .DELTA.Cp for binding were detd. by using isothermal titrn. calorimetry. Ch4D5 Fab and one of the humanized variants (hu4D5-8 Fab) bind p185HER2-ECD with comparable affinity (.DELTA.G.degree. = -1.36 kcal mol-1). The enthalpy changes assocd. with binding, however, are considerably different (ch4D5 Fab .DELTA.H = -17.2 .+-. 1.5 kcal mol-1; hu4D5-8 Fab .DELTA.H = -12.9 .+-. 0.4 kcal mol-1), which suggests a significant difference in the mechanism of antigen binding. This difference may be important for antiproliferative activity since ch4D5 Fab retains activity whereas hu4D5-8 Fab is inactive. Thus, KD measurements alone are insufficient in an attempt to reproduce the activity of a murine antibody in a humanized form. Anal. of the thermodn. data using an empirical method (Sturtevant, J. M., 1977) indicates that differences in the hydrophobic or vibrational contributions to binding cannot account for equiv. .DELTA.G but can account for differing .DELTA.H. The hydrophobic contribution to antigen binding is equiv. for ch4D5 and hu4D5-8 Fab and is consistent with burial of about 960 .ANG.2 of nonpolar surface area upon complex formation.

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ST chimeric antibody p185 ***HER2*** Fab antitumor; antigen binding antibody p185HER2 Fab fragment